Discovery of Novel Small Molecule Mer Kinase Inhibitors for the Treatment of Pediatric Acute Lymphoblastic Leukemia

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Supporting Information

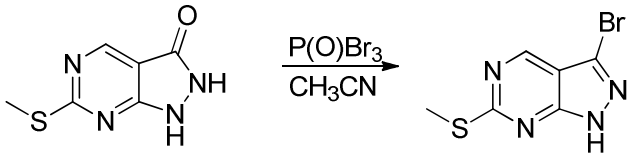
Synthesis of Analogues

Experimental

Microwave reaction was carried out using a CEM Discover-S reactor with a vertically-focused IR external temperature sensor and an Explorer 72 autosampler. The dynamic mode was used to set up the desired temperature and hold time with the following fixed parameters:

PreStirring, 1 min; Pressure, 200 psi; Power, 200 W; PowerMax, off; Stirring, high. Flash chromatography was carried out with pre-packed silica gel disposable columns. Preparative HPLC was performed with the UV detection at 220 or 254 nm. Samples were injected onto a 75 x 30 mm, 5 μM, C18(2) column at room temperature. The flow rate was 30 mL/min. Various linear gradients were used with A being H_2O + 0.5% TFA and B being MeOH. Analytical thin-layer chromatography (TLC) was performed with silica gel 60 F_{254}, 0.25 mm pre-coated TLC plates. TLC plates were visualized using UV_{254} and phosphomolybdic acid with charring. All ^1H NMR spectra were obtained with a 400 MHz spectrometer using $CDCl_3$ (7.26 ppm), DMSO-d_6 (2.50 ppm), or CD_3OD (2.05 ppm) as an internal reference. Signals are reported as m (multiplet), s (singlet), d (doublet), t (triplet), q (quartet), and bs (broad singlet); and coupling constants are reported in Hertz (Hz). ^{13}C NMR spectra were obtained with a 100 MHz spectrometer using $CDCl_3$ (77.2 ppm), DMSO-d_6 (39.5 ppm), or CD_3OD (49.0 ppm) as the internal standard. LC-MS was performed using an analytical instrument with the UV detector set to 220 nm, 254 nm, and 280 nm, and a single quadrupole mass spectrometer using electrospray ionization (ESI) source. Samples were injected (2 μL) onto a 4.6 x 50 mm, 1.8 μM, C18 column at room temperature. A linear gradient from 10% to 100% B (MeOH + 0.1% Acetic Acid) in 5.0 min was followed by pumping 100% B for another 2 or 4 minutes with A being H_2O + 0.1% acetic acid. The flow rate was 1.0 mL/min. High-resolution (positive ion) mass spectra (HRMS) were acquired using a LCMS-TOF mass spectrometer. Analytical HPLC was performed with the UV detection at 321 or 254 nm. Samples were injected onto a 4.6 x 150 mm, 5.0 μm, C18 column at room temperature. The flow rate was 1.0 mL/min. A linear gradient from 10% to 100% B, with A as H_2O + 0.1% TFA and B as MeOH + 0.1% TFA.

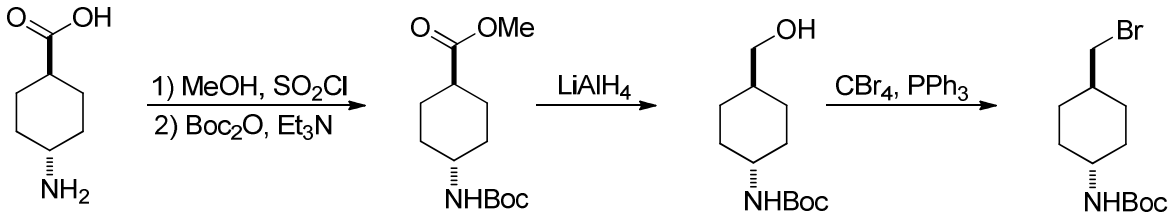
Synthesis of the common intermediates:



2 **3**

3-Bromo-6-(methylthio)-1*H*-pyrazolo[3,4-*d*] pyrimidine (3)

To a suspension of 6-(methylthio)-1*H*-pyrazolo[3,4-*d*] pyrimidin-3(2*H*)-one **2** (2.0 g, 11 mmol) in CH_3CN (180 mL) in a pressure vessel was added a solution of $POBr_3$ (6.3 g, 22 mmol) in CH_3CN. The mixture was sonicated for 30 min before heating at 100 °C for 16 h. After cooling to 0 °C, H_2O and aqueous ammonium hydroxide were added. The aqueous layer was extracted with EtOAc (10×) and the layers were separated. The combined organic layers were dried (Na_2SO_4), and concentrated under reduced pressure. The resulting residue was purified by silica gel column to afford the title compound **3** (1.75 g, 65%) as a white solid. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 14.21 (bs, 1H), 8.98 (s, 1H), 2.56 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 170.5, 155.0, 151.9, 120.5, 110.8, 13.8; LC-MS (ESI+): t_R = 4.882 min, *m/z* 245.0 $[M+1]^+$; HRMS (TOF, ESI+) *m/z*: $[M+H]^+$ calculated for $C_6H_6BrN_4S$, 244.9496; found 244.9499.



45 **46** *trans*-**47**

***trans*-Methyl 4-(*tert*-butoxycarbonylamino)cyclohexanecarboxylate (45)**

To a suspension of *trans*-4-aminocyclohexanecarboxylic acid (5.0 g, 35 mmol) in methanol (50 mL) was added thionyl chloride (2.9 mL, 40 mmol) dropwisely at 0 °C. The white

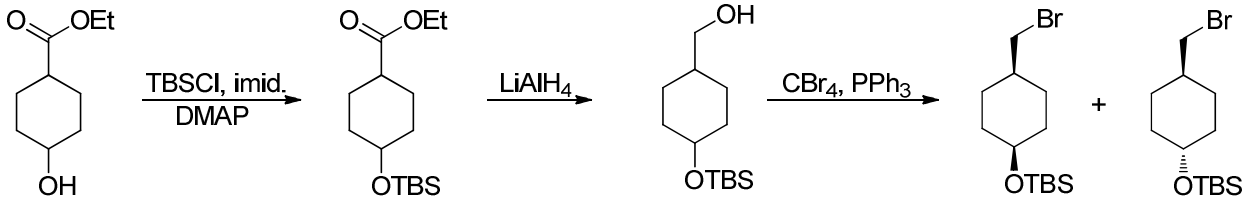
suspension was dissolved. After being stirred at room temperature for 4.5 h, the solution was concentrated and a white solid was obtained. Methylene chloride was added and then evaporated twice to remove trace amount of thionyl chloride. A suspension of the solid in methylene chloride (60 mL) was added triethyl amine (5.4 mL, 38 mmol). A clear solution was obtained. The solution was cooled to 0 °C and Boc anhydride (8.83 mL, 38 mmol) was added slowly. The reaction mixture was stirred at room temperature for 5.0 h, then poured into an aqueous sodium bicarbonate solution. The mixture was extracted with methylene chloride (3×), dried (Na_2SO_4), and concentrated. The crude mixture was purified by silica gel column to provide the title compound **45** (9.0 g, 100%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.37 (bs, 1H), 3.66 (s, 3H), 3.40 (bs, 1H), 2.22 (tt, *J* = 12.1, 3.4 Hz, 1H), 2.03 (dd, *J* = 24.0, 13.2 Hz, 4H), 1.56–1.47 (m, 2H), 1.44 (s, 9H), 1.10 (qd, *J* = 12.7, 3.1 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.0, 155.3, 79.4, 51.8, 49.1, 42.5, 32.7, 28.6, 28.0.

***tert*-Butyl *trans*-4-(hydroxymethyl)cyclohexylcarbamate (46)**

A solution of t*rans*-methyl 4-(*tert*-butoxycarbonylamino)cyclohexanecarboxylate **45** (9.0 g, 35 mmol) in THF (70 mL) was added slowly a 2.0 M solution of $LiAH_4$ in THF (20 mL, 40 mmol) at -78 °C. The reaction was warmed slowly to room temperature (over 4.0 h), and quenched by dropwise addition of water (5.0 mL), followed by addition of NaOH (5 mL) and Na_2SO_4. The mixture was stirred for 20 min and filtered. The filtrate was dried (Na_2SO_4), and concentrated. The crude mixture was passed through a silica gel plug to provide the title compound **46** (5.5 g, contaminated with small amount of impurities) as a white solid and used as such for the next step.

***tert*-Butyl *trans*-4-(bromomethyl)cyclohexylcarbamate (*trans*-47)**

A solution of *tert*-butyl *trans*-4-(hydroxymethyl)cyclohexylcarbamate **46** (5.5 g) and carbon tetrabromide (9.9 g, 30 mmol) in methylene chloride (120 mL) was added triphenylphosphine (7.5 g, 29 mmol) in three portions at 0 °C. The solution was stirred at room temperature for 4.0 h. After evaporation of the solvent, a mixture of EtOAc and hexanes (1:1) was added. The resulting white solid was filtered off. The filtrate was concentrated and purified by silica gel column to provide the title compound *trans*-**47** (6.5 g, 64% (over 2 steps)) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 3.32 (d, J = 6.4 Hz, 2H), 3.30–3.19 (m, 1H), 1.93 (d, J = 9.6 Hz, 4H), 1.63–1.50 (m, 1H), 1.43 (s, 9H), 1.29–1.05 (m, 4H); ^{13}C NMR (100 MHz, CD_3OD) δ 157.8, 79.8, 50.8, 40.7, 40.3 , 33.4, 31.4, 28.8. ***tert*-Butyl *cis*-4-(bromomethyl) cyclohexylcarbamate (*cis*-47)** (5.2 g, 51%) was prepared as a white solid according to the procedure for *trans*-**47** from *cis*-4-aminocyclohexanecarboxylic acid (5.0 g, 35 mmol)**.** ^{1}H NMR (400 MHz, CD_3OD) δ 3.65–3.53 (m, 1H), 3.38 (d, J = 6.8 Hz, 2H), 1.78–1.52 (m, 7H), 1.78–1.52 (dd, J = 9.0, 3.6 Hz, 2H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD) δ 157.8, 79.9, 47.9, 39.7, 39.2, 30.1, 28.8, 27.4. ***tert*-Butyl 4-(bromomethyl)cyclohexylcarbamate (47)** (5.4 g, 53%) was prepared as a white solid according to the procedure for *trans*-**47** from 4-aminocyclohexanecarboxylic acid (5.0 g, 35 mmol)**.**



48 **49** *cis*-**50** *trans*-**50**

Ethyl 4-(*tert*-butyldimethylsilyloxy)cyclohexanecarboxylate (48)

To a solution of ethyl 4-hydroxycyclohexanecarboxylate (2.42 mL, 15 mmol) in DMF (10 mL) were added imidazole (1.23 g, 18 mmol) and 4-dimethylaminopyridine (0.37 g, 3.0 mmol). The resulting solution was cooled to 0 °C and *tert*-butylchlorodimethylsilane (2.71 g, 18 mmol) was added slowly. The reaction was warmed to room temperature and stirred for 16 h. The reaction was quenched with saturated aqueous NH_4Cl and extracted with methylene chloride (3×). The combined organic layers were dried (Na_2SO_4), concentrated and purified by silica gel column to provide the title compound **48** (*cis*:*trans* = 2:1) as a white solid (4.09 g, 100%).

4-(*tert*-Butyldimethylsilyloxy)cyclohexyl)methanol (49)

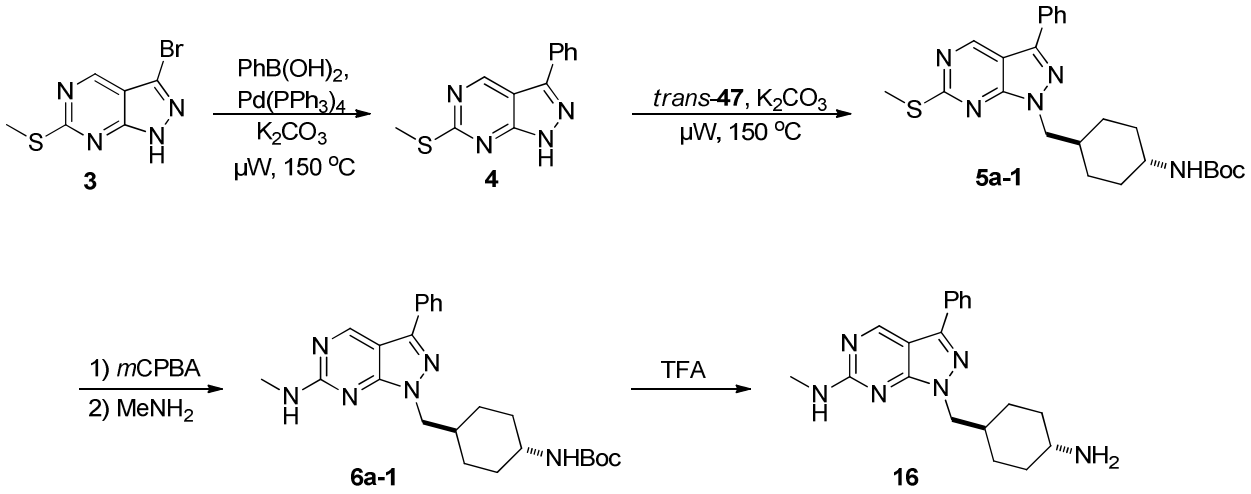
To a 100 mL flask were added **48** (4.09 g, 15 mmol) and THF (30 mL). A 2.0M solution of lithium aluminum hydride in THF (8.3 mL, 16.5 mmol) was added dropwisely at -78 °C. The reaction was slowly warmed to room temperature over 3.0 h. Minimum amount of water was added slowly to quench the reaction followed by addition of a 1.0 N aqueous solution of NaOH and Na_2SO_4. The mixture was stirred for 20 min until the solution portion is clear. The mixture was filtered. The filtrate was concentrated to yield the title compound **49** (3.67 g) and used as such in the next step. ***trans*-(*tert*-Butyldimethylsilyloxy)cyclohexyl)methanol** (*trans*-**49)** (2.93 g, 80%) was prepared as a white solid according to the procedure for **49** from *trans*-ethyl 4-hydroxycyclohexanecarboxylate (2.58 g, 15 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.52 (tt, *J* = 10.7, 4.3 Hz, 1H), 3.44 (d, *J* = 6.3 Hz, 2H), 1.94–1.84 (m, 2H), 1.84–1.74 (m, 2H), 1.49–1.37 (m, 1H), 1.37–1.24 (m, 3H), 1.05–0.92 (m, 2H), 0.88 (s, 9H), 0.05 (s, 6H).

4-(Bromomethyl)cyclohexyloxy)(*tert*-butyl)dimethylsilane (50)

To a solution of **49** (3.67 g, 15 mmol) in methylene chloride (75 mL) was added carbon tetrabromide (6.27 g, 18.9 mmol). Triphenyl phosphine (4.41 g, 16.8 mmol) was added in three

portions at 0 °C. The reaction was stirred at room temperature for 4.0 h. The volume of the solution was concentrated until about 10 mL. 50% Ethyl acetate in hexanes was added. After removal of participates, the filtrate was condensed and purified by silica gel column to provide *cis*-4-(*tert*-butyldimethylsilyloxy)cyclohexyl)methanol *cis*-**50** (2.5 g, 54% (over 2 steps)) and *trans*-4-(*tert*-butyldimethylsilyloxy)cyclohexyl)methanol *trans*-**50** (1.1g, 24% (over 2 steps)) as a colorless oil. Data for *cis*-**50:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.97–3.91 (m, 1H), 3.29 (d, J = 6.5 Hz, 2H), 1.71–1.57 (m, 5H), 1.55–1.40 (m, 4H), 0.89 (s, 9H), 0.03 (s, 6H). Data for *trans*-**50:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.58–3.47 (m, 1H), 3.28 (d, J = 6.2 Hz, 2H), 1.93–1.82 (m, 4H), 1.67–1.55 (m, 1H), 1.38–1.24 (m, 2H), 1.15–1.00 (m, 2H), 0.88 (s, 9H), 0.05 (s, 6H).

An Example for Path a in Scheme 1:



6-(Methylthio)-3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidine (4) (General Procedure A)

A 10 mL microwave tube was charged with **3** (0.049 g, 0.20 mmol), K_2CO_3 (0.083 g, 0.6 mmol), phenylboronic acid (0.037 g, 0.30 mmol), $Pd(PPh_3)_4$ (0.023 g, 0.020 mmol), THF (2.0 mL) and H_2O (1.0 mL). The resulting mixture was heated at 150 °C for 10 min. After cooling to room temperature, the reaction was diluted with EtOAc and THF (1:1). The mixture was washed with brine. The aqueous phase was extracted with EtOAc and THF (1:1) (5×). The combined

organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column to provide the title compound **4** (0.046 g, 95%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.45 (s, 1H), 8.04 (dt, J = 7.2, 1.2 Hz, 2H), 7.54 (tt, J = 10.2, 4.6 Hz, 2H), 7.46 (tt, J = 7.2, 1.2 Hz, 1H), 2.59 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.7, 155.8, 152.9, 143.9, 131.9, 129.1, 129.0, 126.7, 108.3, 13.7; LC-MS (ESI+): t_R = 5.783 min, *m/z* 243.15 $[M+1]^+$.

***tert*-Butyl *trans*-4-((6-(methylthio)-3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl) cyclohexylcarbamate (5a-1)** (General Procedure B)

A microwave tube was charged with **4** (0.073 g, 0.30 mmol), potassium carbonate (0.12 g, 0.90 mmol), and DMSO (1.0 mL). The resulting mixture was stirred for 20 min, then was added a solution of **47** (0.10 g, 0.36 mmol) in THF (2.0 mL). The resulting mixture was heated at 150 °C for 10 min in microwave. The reaction mixture was poured into water and extracted with Et_2O (3×). The combined ether layer was dried (Na_2SO_4) and concentrated. The crude mixture was purified by silica gel column to provide the title compound **5a-1** (0.12 g, 88%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (s, 1H), 7.97–7.89 (m, 2H), 7.56–7.48 (m, 2H), 7.45 (tt, J = 7.3, 1.2 Hz, 1H), 4.35 (bs, 1H), 4.30 (d, J = 7.1 Hz, 2H), 3.40 (bs, 1H), 2.65 (s, 3H), 2.10–1.95 (m, 3H), 1.71 (d, J = 12.1 Hz, 2H), 1.43 (s, 9H), 1.23 (dd, J = 24.4, 10.8 Hz, 2H), 1.07 (ddd, J = 25.4, 12.7, 2.9 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 155.3, 154.8, 152.3, 144.3, 132.3, 129.3, 127.2, 109.4, 79.3, 52.5, 49.7, 37.7, 32.9, 29.6, 28.6, 14.5.

***tert*-Butyl t*rans*-4-((6-(methylamino)-3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl) methyl)cyclohexylcarbamate (6a-1)** (General Procedure C)

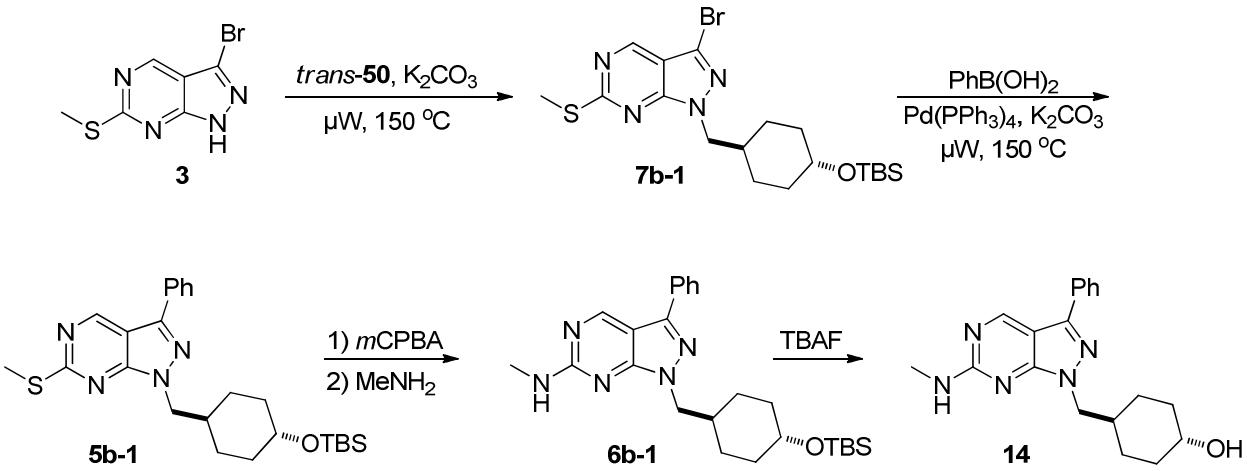
To a solution of **5a-1** (0.14 g, 0.3 mmol) in methylene chloride (5.0 mL) was added *meta*-chloroperoxybenzoic acid (0.20 g, 77%, 0.90 mmol) at room temperature. After stirring at room

temperature for 2.0 h, the light purple solution was quenched with a 1.0 N aqueous solution of NaOH. The aqueous layer was extracted with EtOAc (3×). The organic layers were combined, dried (Na_2SO_4), and concentrated. The residue was dissolved in THF (1.5 mL), followed by the addition of a 2.0 M methylamine solution in THF (1.5 mL, 3.0 mmol). The resulting solution was heated at 60 °C for 2.0 h, cooled to room temperature, and concentrated. The crude mixture was purified by silica gel column to provide the title compound **6a-1** (0.10 g, contaminated with small amount of impurities) as a white solid.

1-((*trans*-4-Aminocyclohexyl)methyl)-*N*-methyl-3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-6-amine (16) (General Procedure D)

To a solution of **6a-1** (0.082 g, 0.19 mmol) in methylene chloride (3.0 mL) was added trifluoroacidic acid (0.60 mL). The reaction mixture was stirred at room temperature for 2.0 h, concentrated and basified by a 7.0 M aqueous solution of ammonia to pH 12. After evaporation, the residue was purified by silica gel column to provide the title compound **16** (0.051 g, 61% (over 2 steps)) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.95 (s, 1H), 7.94–7.83 (m, 2H), 7.55–7.44 (m, 2H), 7.44–7.36 (m, 1H), 4.16 (d, J = 7.5 Hz, 2H), 3.06–2.91 (m, 1H), 2.99 (s, 3H), 2.13–1.93 (m, 3H), 1.78 (d, J = 12.7 Hz, 2H), 1.45–1.14 (m, 4H); ^{13}C NMR (100 MHz, CD_3OD) δ 162.9, 157.7, 154.9, 145.7, 133.6, 130.01, 129.95, 128.0, 106.6, 52.3, 51.3, 38.4, 31.9, 29.7, 28.5; LC-MS (ESI+): t_R = 4.042 min, *m/z* 337.3 $[M+1]^+$.

An Example for Path b in Scheme 1:



3-Bromo-1-((*trans*-4-(*tert*-butyldimethylsilyloxy)cyclohexyl)methyl)-6-(methylthio)-1*H*-pyrazolo[3,4-d]pyrimidine (7b-1)

The title compound **7b-1** (0.31 g, 82%) was prepared as a white solid according to general procedure B from **3** (0.20 g, 0.80 mmol) and *trans*-**50** (0.31 g, 1.0 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (s, 1H), 4.22 (d, *J* = 7.0 Hz, 2H), 3.59–3.45 (m, 1H), 2.62 (s, 3H), 2.05–1.91 (m, 1H), 1.84 (d, *J* = 12.8 Hz, 2H), 1.63 (d, *J* = 12.8 Hz, 2H), 1.35–1.21 (m, 2H), 1.15–1.00 (m, 2H), 0.86 (s, 9H), 0.03 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 154.4, 151.7, 120.1, 111.7, 71.4, 52.8, 37.4, 35.1, 28.9, 26.0, 18.3, 14.6, -4.5.

1-((*trans*-4-(tert-Butyldimethylsilyloxy)cyclohexyl)methyl)-6-(methylthio)-3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidine (5b-1)

The title compound **5b-1** (0.079 g, 84%) was prepared as a white solid according to general procedure A from **7b-1** (0.094 g, 0.20 mmol) and phenylboronic acid (0.037 g, 0.30 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (s, 1H), 7.98–7.91 (m, 2H), 7.56–7.49 (m, 2H), 7.48–7.41 (m, 1H), 4.30 (d, *J* = 7.0 Hz, 2H), 3.59–3.46 (m, 1H), 2.66 (s, 3H), 2.13–1.98 (m, 1H), 1.91–1.81 (m, 2H), 1.74–1.63 (m, 2H), 1.34–1.24 (m, 2H), 1.20–1.07 (m, 2H), 0.87 (s, 9H), 0.04 (s, 6H); LC-MS (ESI+): t_R = 7.371 min, *m/z* 469.0 $[M+1]^+$.

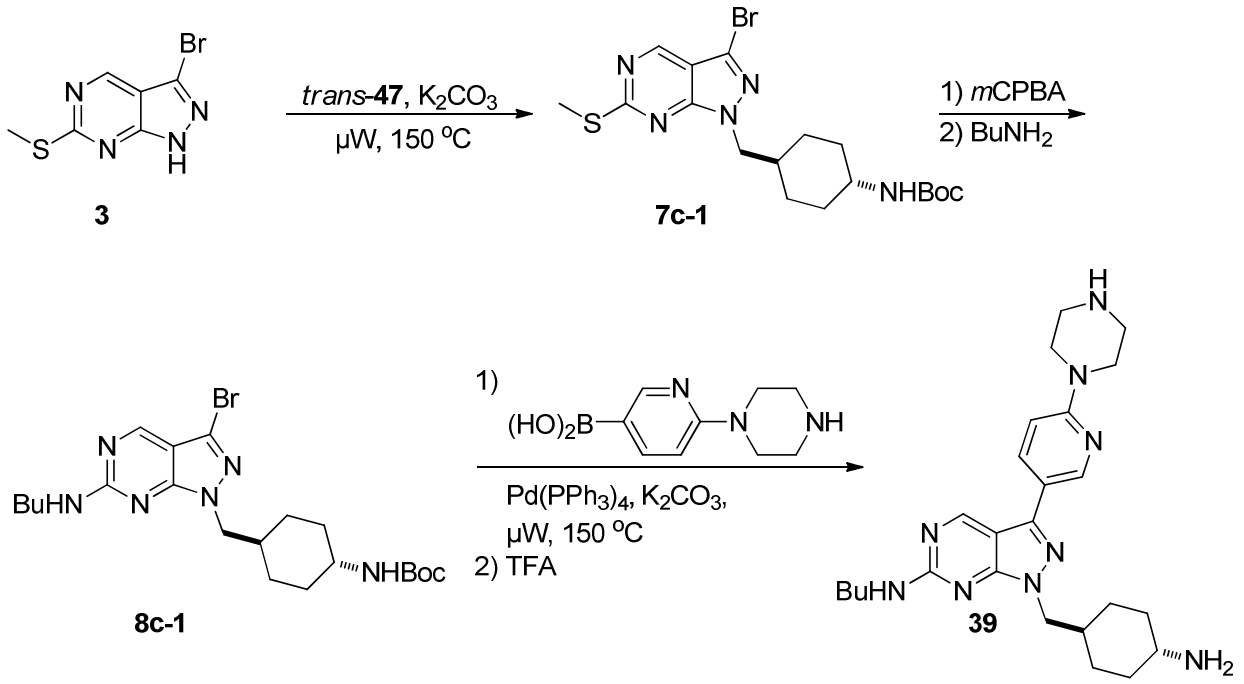
1-((*trans*-4-(*tert*-Butyldimethylsilyloxy)cyclohexyl)methyl)-*N*-methyl-3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-6-amine (6b-1)

The title compound **6b-1** (0.055 g, contaminated with small amount of impurities) was prepared as a white solid according to general procedure C from **5b-1** (0.079 g, 0.17 mmol). LC-MS (ESI+): t_R = 6.403 min, *m/z* 452.0 $[M+1]^+$.

***trans*-4-((6-(Methylamino)-3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methylcyclohexanol (14)** (General Procedure E)

To a solution of **6b-1** (0.055 g, 0.12 mmol) in THF (3.0 mL) was added a 1.0 M THF solution of TBAF (1.0 mL, 1.0 mmol). The reaction mixture was refluxed for 2.0 h, concentrated and purified by reverse phase HPLC to provide the title compound **14** (0.033 g, 59% (over 2 steps)) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 9.09 (s, 1H), 7.95 (d, *J* = 7.4 Hz, 2H), 7.59–7.42 (m, 3H), 4.21 (d, *J* = 7.0 Hz, 2H), 3.56–3.46 (m, *J* = 3.7 Hz, 1H), 3.06 (s, 3H), 2.11–1.90 (m, 3H), 1.73 (d, *J* = 11.1 Hz, 2H), 1.30–1.14 (m, 4H); LC-MS (ESI+): t_R = 5.435 min, *m/z* 338.2 $[M+1]^+$.

An Example for Path c in Scheme 1:



***tert*-Butyl *trans*-4-((3-bromo-6-(methylthio)-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclohexylcarbamate (7c-1)**

The title compound **7c-1** (0.34 g, 93%) was prepared as a white solid according to general procedure B from **3** (0.20 g, 0.80 mmol) and *trans*-**47** (0.29 g, 1.0 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (s, 1H), 4.55 (bs, 1H), 4.12 (d, J = 7.1 Hz, 2H), 3.29 (bs, 1H), 2.51 (s, 3H), 1.94–1.81 (m, 2H), 1.63–1.51 (m, 2H), 1.32 (s, 9H), 1.13–0.93 (m, 4H). LC-MS (ESI+): t_R = 5.802 min, *m/z* 456.1 $[M+1]^+$.

***tert*-Butyl *trans*-4-((3-bromo-6-(butylamino)-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclohexylcarbamate (8c-1)**

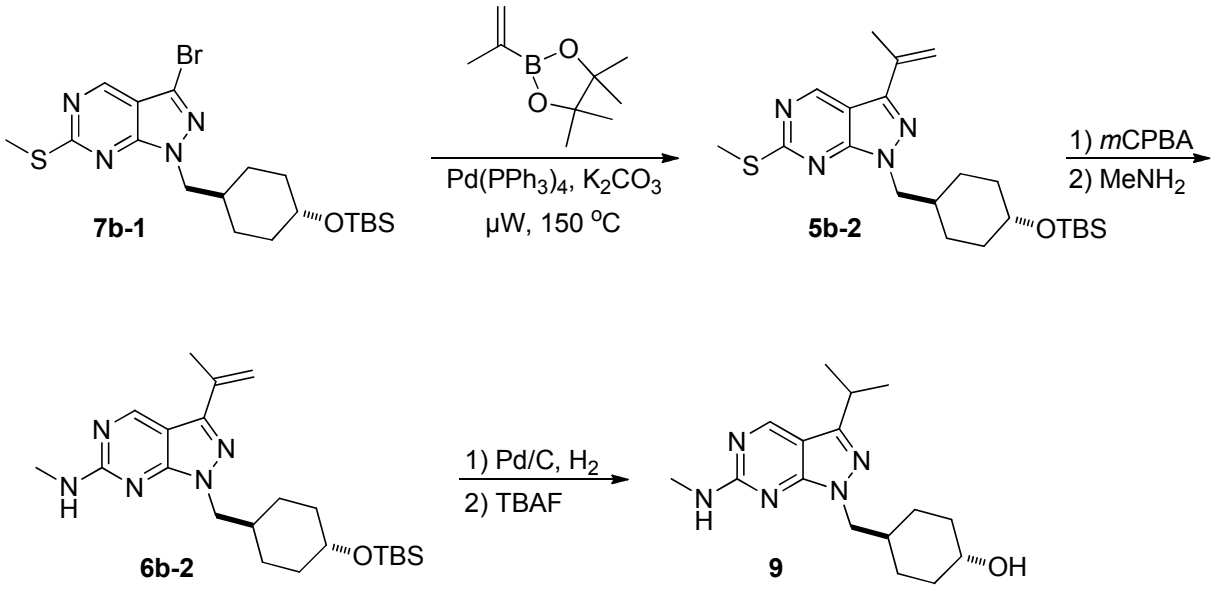
The title compound **8c-1** (0.41 g, 78%) was prepared as a white solid according to general procedure C from **7c-1** (0.46 g, 1.1 mmol) and *n*-butylamine (0.49 mL, 5.0 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (s, 1H), 5.37 (bs, 1H), 4.35 (bs, 1H), 4.06 (d, J = 7.0 Hz, 2H), 3.46 (dd, J = 13.1, 6.8 Hz, 2H), 3.39 (bs, 1H), 2.03–1.88 (m, 3H), 1.72–1.58 (m, 4H), 1.43 (s,

9H), 1.23–1.01 (m, 4H), 0.97 (t, J = 7.3 Hz, 3H). LC-MS (ESI+): t_R = 6.298 min, *m/z* 481.2 $[M+1]^+$.

1-((*trans*-4-Aminocyclohexyl)methyl)-*N*-butyl-3-(6-(piperazin-1-yl)pyridin-3-yl)-1*H*-pyrazolo[3,4-d]pyrimidin-6-amine (39) (General Procedure F)

To a microwave tube was charge with **8c-1** (0.099 g, 0.21 mmol), 6-(piperazin-1-yl)pyridin-3-ylboronic acid (0.060 g, 0.21 mmol), potassium carbonate (0.091 g, 0.66 mmol), tetrakis(triphenylphosphine) palladium (0.040 g, 0.035 mmol), THF (2.0 mL) and water (0.50 mL). The mixture was heated in microwave at 150 °C for 10 min. The reaction mixture was poured into water. And the aqueous layer was extracted with Et_2O (3×). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by silica gel column. The intermediate was dissolved in methylene chloride (2.0 mL), followed by the addition of trifluoroacidic acid (0.50 mL). The reaction mixture was stirred at room temperature for 2.0 h. After concentration, the residue was purified by reverse phase prep-HPLC to provide the title compound **39** (0.092 g, 59%) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 9.16 (s, 1H), 8.76 (d, J = 2.3 Hz, 1H), 8.23 (dd, J =2.4, 9.0 Hz, 1H), 7.11 (d, J = 9.0 Hz, 1H), 4.02-3.90 (m, 4H), 3.54 (t, J = 7.1 Hz, 2H), 3.40–3.34 (m, 4H), 3.13–3.02 (m, 1H), 2.15-2.01 (m, 3H), 1.87 (d, J = 11.7 Hz, 2H), 1.75–1.65 (m, 2H), 1.55–1.20 (m, 6H), 1.01 (t, J = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, cd_3od) δ 163.0, 159.6, 157.4, 152.9, 147.1, 144.0, 137.6, 120.4, 109.0, 106.5, 52.4, 51.3, 44.3, 43.4, 42.1, 38.2, 32.4, 31.2, 29.6, 21.2, 14.2; LC-MS (ESI+): t_R = 3.558 min, *m/z* 464.3 $[M+1]^+$; HRMS (TOF, ESI) *m/z*: $[M+H]^+$ calculated for $C_{25}H_{38}N_9$: 464.3250, found: 464.3268; HPLC: t_R = 6.905 min; purity: 96%.

Synthesis of Compounds 9-17 (Table 1):



1-((*trans*-4-(tert-Butyldimethylsilyloxy)cyclohexyl)methyl)-6-(methylthio)-3-(prop-1-en-2-yl)-1*H*-pyrazolo[3,4-d]pyrimidine (5b-2)

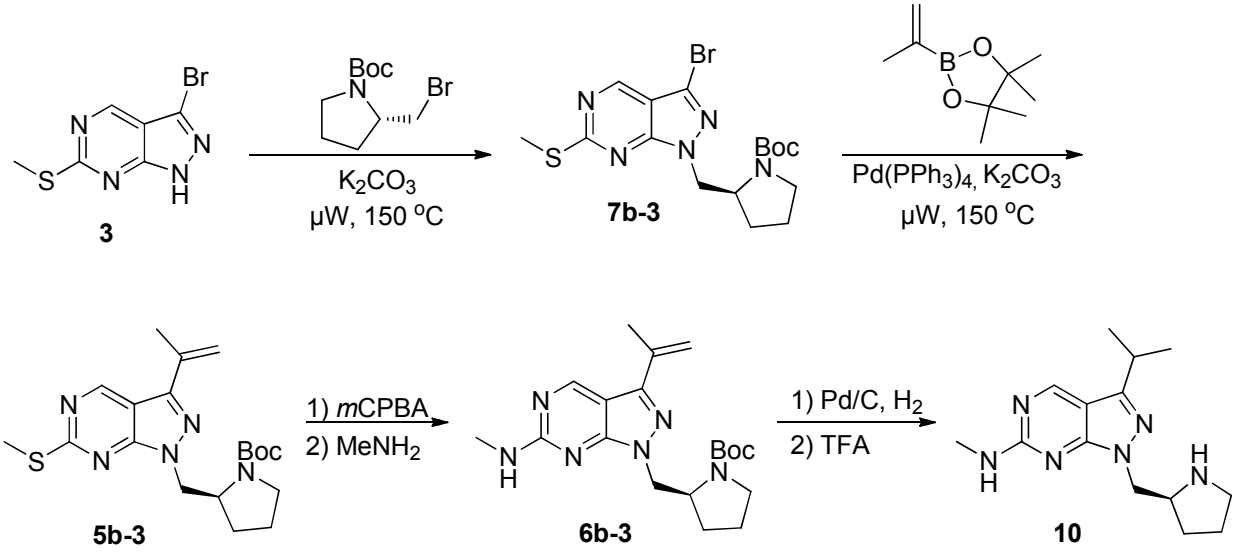
The title compound **5b-2** (0.061 g, 71%) was prepared as a white solid according to general procedure A from **7b-1** (0.094 g, 0.20 mmol) and isopropenylboronic acid pinacol ester (0.050 g, 0.30 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.04 (s, 1H), 5.76 (s, 1H), 5.46–5.37 (m, 1H), 4.22 (d, J = 7.1 Hz, 2H), 3.60–3.46 (m, 1H), 2.63 (s, 3H), 2.27 (s, 3H), 2.04–1.92 (m, 1H), 1.90–1.80 (m , 2H), 1.63 (d, J = 13.1 Hz, 2H), 1.32–1.21 (m, 2H), 1.17– 1.05 (m, 2H), 0.87 (s, 9H), 0.03 (s, 6H); LC-MS (ESI+): t_R = 6.891 min, *m/z* 433.0 $[M+1]^+$.

1-((*trans*-4-(tert-Butyldimethylsilyloxy)cyclohexyl)methyl)-*N*-methyl-3-(prop-1-en-2-yl)-1*H*-pyrazolo[3,4-d]pyrimidin-6-amine (6b-2)

The title compound **6b-2** (0.045 g, 76%) was prepared as a white solid according to general procedure C from **5b-2** (0.061 g, 0.14 mmol). LC-MS (ESI+): t_R = 7.142 min, *m/z* 416.0 $[M+1]^+$.

***trans*-4-((3-Isopropyl-6-(methylamino)-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl) cyclohexanol (9)**

To a 25 mL round bottom flask was charged with **6b-2** (0.10 g, 0.24 mmol), EtOH (5.0 mL), and Pd/C (0.010 g, 10 wt%). The flask was vacuumed and refilled with H_2 (3×). The reaction was stirred for 4.0 h under H_2 balloon pressure. The reaction mixture was filtered through Celite plug and concentrated. The residue was dissolved in THF (3.0 mL). After addition of TBAF (1.0 mL, 1.0 M in THF, 1.0 mmol), the reaction mixture was refluxed for 3.0 h. After concentration, the residue was purified with reverse phase HPLC to provide the title compound **9** (0.055 g, 76%) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.74 (s, 1H), 4.05 (d, J = 7.1 Hz, 2H), 3.54–3.42 (m, 1H), 3.23 (heptet, J = 7.0 Hz, 1H), 2.97 (s, 3H), 1.99–1.84 (m, 3H), 1.62 (d, J = 12.6 Hz, 2H), 1.40 (d, J = 7.0 Hz, 6H), 1.27–1.05 (m, 4H); LC-MS (ESI+): t_R = 4.953 min, *m/z* 304.2 $[M+1]^+$.



(*S*)-*tert*-Butyl 2-((3-bromo-6-(methylthio)-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)pyrrolidine-1-carboxylate (7b-3)

The title compound **7b-3** (0.30 g, 87%) was prepared as a white solid according to general procedure B from **3** (0.20 g, 0.80 mmol) and (*S*)-tert-butyl 2-(bromomethyl)pyrrolidine-1-carboxylate (0.26 g, 1.0 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (s, 1H), 4.58–4.21 (m, 3H), 3.50–3.17 (dd, *J* = 25.6, 17.7 Hz, 2H), 2.60 (s, 3H), 1.94–1.62 (m, 4H), 1.38 (s, 9H); LC-MS (ESI+): t_R = 5.597 min, *m/z* 428.1 $[M+1]^+$.

(S)-*tert*-Butyl 2-((6-(methylthio)-3-(prop-1-en-2-yl)-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)pyrrolidine-1-carboxylate (5b-3)

The title compound **5b-3** (0.058 g, 74%) was prepared as a white solid according to general procedure A from **7b-3** (0.086 g, 0.20 mmol) and isopropenylboronic acid pinacol ester (0.050 g, 0.30 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.05 (s, 1H), 5.75 (s, 1H), 5.41 (s, 1H), 4.69–4.21 (m, 3H), 3.47–3.20 (m, 2H), 2.63 (s, 3H), 2.25 (s, 3H), 1.94–1.67 (m, 4H), 1.41 (s, 9H); LC-MS (ESI+): t_R = 6.182 min, *m/z* 390.2 $[M+1]^+$.

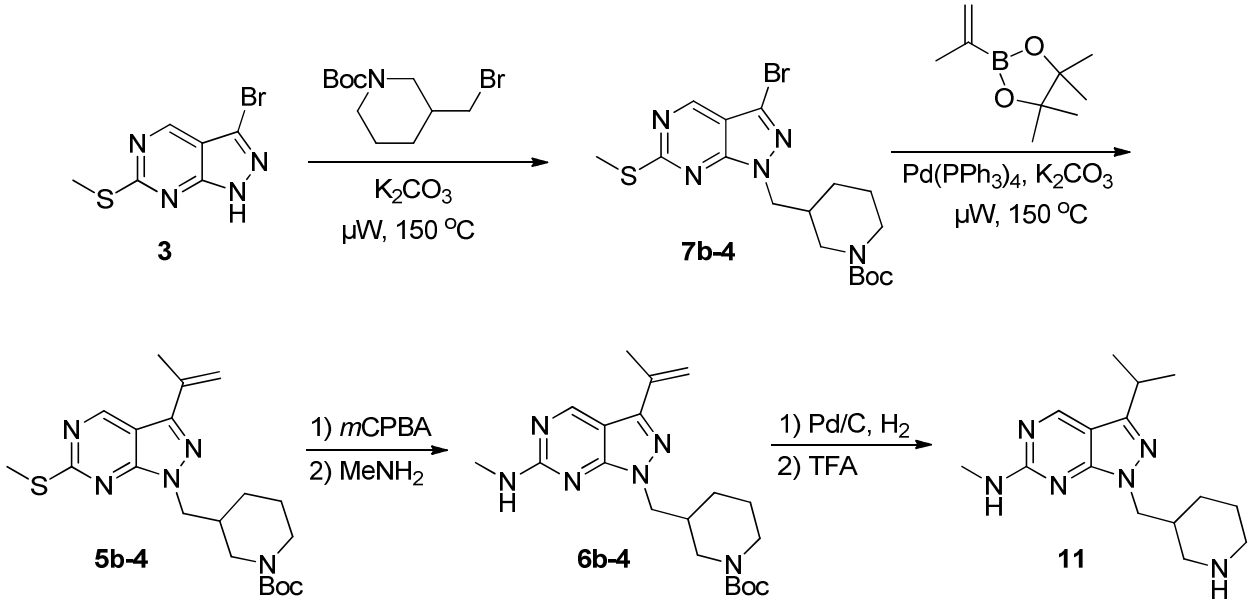
(*S*)-*tert*-Butyl 2-((6-(methylamino)-3-(prop-1-en-2-yl)-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)pyrrolidine-1-carboxylate (6b-3)

The title compound **6b-3** (0.039 g, 70%) was prepared as a white solid according to general procedure C from **5b-3** (0.058 g, 0.15 mmol). LC-MS (ESI+): t_R = 5.896 min, *m/z* 373.3 $[M+1]^+$.

(*S*)-3-Isopropyl-*N*-methyl-1-(pyrrolidin-2-ylmethyl)-1*H*-pyrazolo[3,4-d]pyrimidin-6-amine (10) (General Procedure G)

To a 25 mL round bottom flask was charged with **6b-3** (0.10 g, 0.27 mmol), EtOH (5.0 mL), and Pd/C (0.010 g, 10 wt%). The flask was vacuumed and refilled with H_2 (3×). The reaction was stirred for 4.0 h under H_2 balloon pressure. The reaction mixture was filtered through Celite plug and concentrated. The residue was dissolved in 2.0 mL CH_2Cl_2. After

addition of TFA (0.50 mL), the reaction mixture was stirred for 3.0 h at room temperature. After concentration, the residue was purified with reverse phase HPLC to provide the title compound **10** (0.061 g, 82%) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 9.00 (s, 1H), 4.67 (dd, *J* = 15.0, 3.8 Hz, 1H), 4.50 (dd, *J* = 15.0, 8.4 Hz, 1H), 4.16-4.02 (m, 1H), 3.48–3.31 (m, 3H), 3.05 (d, *J* = 1.1 Hz, 3H), 2.37-2.23 (m, 1H), 2.16-1.98 (m, 2H), 1.93-1.80 (m, 1H), 1.41 (d, *J* = 7.0 Hz, 6H); LC-MS (ESI+): t_R = 2.967 min, *m/z* 275.2 $[M+1]^+$.



***tert*-Butyl 3-((3-bromo-6-(methylthio)-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-1-carboxylate (7b-4)**

The title compound **7b-4** (0.030 g, 84%) was prepared as a white solid according to general procedure B from **3** (0.20 g, 0.80 mmol) and (±)-tert-butyl 3-(bromomethyl)piperidine-1-carboxylate (0.28 g, 1.0 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (s, 1H), 4.32–4.23 (m, 2H), 3.99–3.66 (bs, 1H), 3.83 (dt, *J* = 13.1, 4.0 Hz, 1H), 2.96–2.82 (m, 1H), 2.75 (bs, 1H), 2.62 (s, 3H), 2.31–2.17 (m, 1H), 1.76–1.67 (m, 2H), 1.40 (s, 10H), 1.28–1.16 (m, 1H); LC-MS (ESI+): t_R = 5.799 min, *m/z* 442.0 $[M+1]^+$.

***tert*-Butyl 3-((6-(methylthio)-3-(prop-1-en-2-yl)-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-1-carboxylate (5b-4)**

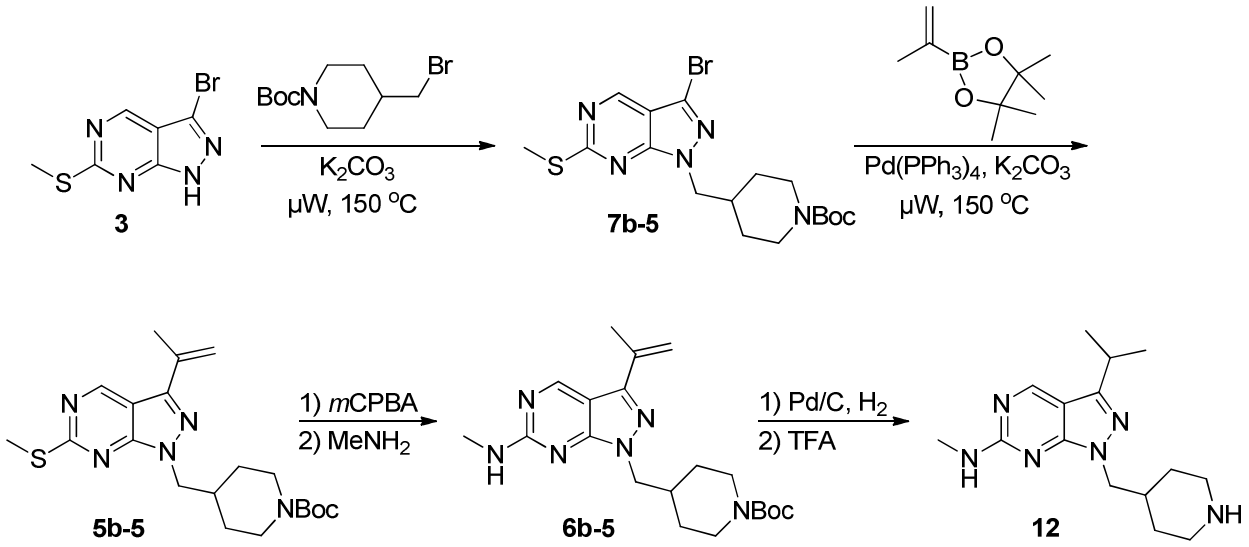
The title compound **5b-4** (0.056 g, 69%) was prepared as a white solid according to general procedure A from **7b-4** (0.088 g, 0.20 mmol) and isopropenylboronic acid pinacol ester (0.050 g, 0.030 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.03 (s, 1H), 5.74 (s, 1H), 5.1 (t, *J* = 1.2 Hz ,1H), 4.35–4.19 (m, 2H),3.89–3.72 (m, 1H), 3.83 (dt, *J* = 13.0, 3.9 Hz, 1H), 2.89–2.80 (m, 1H), 2.79–2.67 (m, 1H), 2.61 (s, 3H), 2.25 (s, 3H), 2.28–2.16 (m, 1H), 1.77–1.62 (m, 2H), 1.38 (s, 10H), 1.25–1.13 (m, 1H); LC-MS (ESI+): t_R = 6.001 min, *m/z* 404.2 $[M+1]^+$.

***tert*-Butyl 3-((6-(methylamino)-3-(prop-1-en-2-yl)-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-1-carboxylate (6b-4)**

The title compound **6b-4** (0.040 g, 74%) was prepared as a white solid according to general procedure C from **5b-4** (0.056 g, 0.14 mmol). LC-MS (ESI+): t_R = 5.992 min, *m/z* 387.3 $[M+1]^+$.

3-Isopropyl-*N*-methyl-1-(piperidin-3-ylmethyl)-1*H*-pyrazolo[3,4-d]pyrimidin-6-amine (11)

The title compound **11** (0.023 g, 79%) was prepared as a yellow solid according to general procedure G from **6b-4** (0.040 g, 0.10 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 8.97 (s, 1H), 4.31–4.16 (m, 2H), 3.35-3.25 (m, 2H), 3.05 (s, 3H), 2.96–2.81 (m, 2H), 2.44 (bs, 1H), 1.93–1.73 (m, 2H), 1.72-1.57 (m, 1H), 1.45–1.32 (m, 1H), 1.48–1.24 (m, 1H), 1.39 (d, *J* =6.8 Hz, 6H); LC-MS (ESI+): t_R = 3.268 min, *m/z* 289.2 $[M+1]^+$.



***tert*-Butyl 4-((3-bromo-6-(methylthio)-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-1-carboxylate (7b-5)**

The title compound **7b-5** (0.31 g, 87%) was prepared as a white solid according to general procedure B from **3** (0.20 g, 0.80 mmol) and tert-butyl 4-(bromomethyl)piperidine-1-carboxylate (0.28 g, 1.0 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (s, 1H), 4.26 (d, J = 7.0 Hz, 2H), 4.14–4.01 (m, 2H), 2.75–2.64 (m, 2H), 2.61 (s, 3H), 2.24–2.10 (m, 1H), 1.59–1.50 (m, 3H), 1.43 (s, 9H), 1.30–1.18 (m, 2H); LC-MS (ESI+): t_R = 6.114 min, *m/z* 464.1 $[M+Na]^+$.

***tert*-Butyl 4-((6-(methylthio)-3-(prop-1-en-2-yl)-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-1-carboxylate (5b-5)**

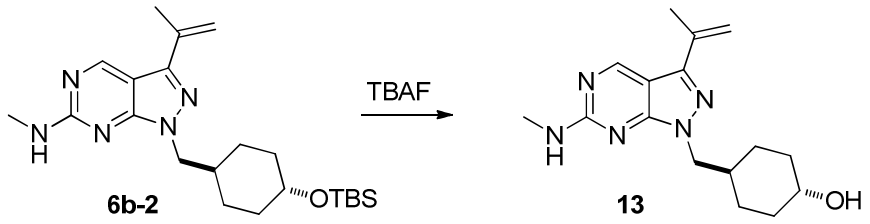
The title compound **5b-5** (0.052 g, 64%) was prepared as a white solid according to general procedure A from **7b-5** (0.088 g, 0.20 mmol) and isopropenylboronic acid pinacol ester (0.050 g, 0.30 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.05 (s, 1H), 5.76 (s, 1H), 5.42 (t J = 1.0 Hz, 1H), 4.28 (d, J = 7.1 Hz, 2H), 4.09 (bs, 2H), 2.68 (t, J = 15.5, 2H), 2.63 (s, 3H), 2.26 (s, 3H), 2.24–2.12 (m, 1H), 1.56 (d, , J = 16.0 Hz, 2H), 1.45 (s, 9H), 1.32–1.19 (m, 2H); LC-MS (ESI+): t_R = 6.326 min, *m/z* 404.2 $[M+1]^+$.

***tert*-Butyl 4-((6-(methylamino)-3-(prop-1-en-2-yl)-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)piperidine-1-carboxylate (6b-5)**

The title compound **6b-5** (0.033 g, 67%) was prepared as a white solid according to general procedure C from **5b-5** (0.052 g, 0.13 mmol).

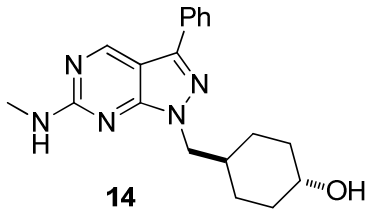
3-Isopropyl-*N*-methyl-1-(piperidin-4-ylmethyl)-1*H*-pyrazolo[3,4-d]pyrimidin-6-amine (12)

The title compound **12** (0.022 g, 90%) was prepared as a yellow solid according to general procedure G from **6b-5** (0.033 g, 0.086 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 9.02 (s, 1H), 4.24 (d, *J* = 6.8 Hz, 2H), 3.47–3.37 (m, 2H), 3.35–3.25 (m, 1H), 3.07 (s, 3H), 3.04–2.94 (m, 2H), 2.41–2.28 (m, 1H), 1.91 (d, *J* = 13.8 Hz, 2H), 1.64–1.51 (m, 2H), 1.40 (d, *J* = 7.0 Hz, 6H); LC-MS (ESI+): t_R = 2.991 min, *m/z* 289.2 $[M+1]^+$.



***trans*-4-((6-(Methylamino)-3-(prop-1-en-2-yl)-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclohexanol (13)**

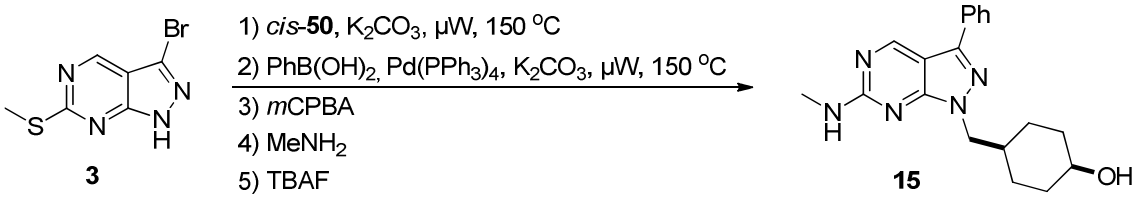
The title compound **13** (0.027 g, 80%) was prepared as a yellow solid according to general procedure E from **6b-2** (0.045 g, 0.11 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 8.95 (s, 1H), 5.79 (s, 1H), 5.47 (s, 1H), 4.13 (d, *J* = 7.0 Hz, 2H), 3.56–3.42 (m, 1H), 3.03 (s, 3H), 2.21 (s, 3H), 1.95 (d, *J* = 11.5 Hz, 3H), 1.68 (d, *J* = 12.0 Hz, 2H), 1.30–1.07 (m, 4H); LC-MS (ESI+): t_R = 5.219 min, *m/z* 302.2 $[M+1]^+$.



14

***trans*-4-((6-(Methylamino)-3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclohexanol (14)**

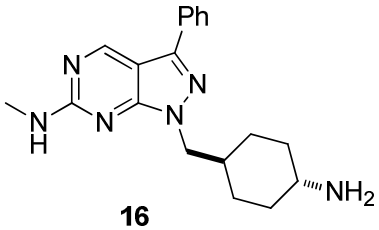
See 'An Example for Path b in Scheme 1'.



3 **15**

***cis*-4-((6-(Methylamino)-3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclohexanol (15)**

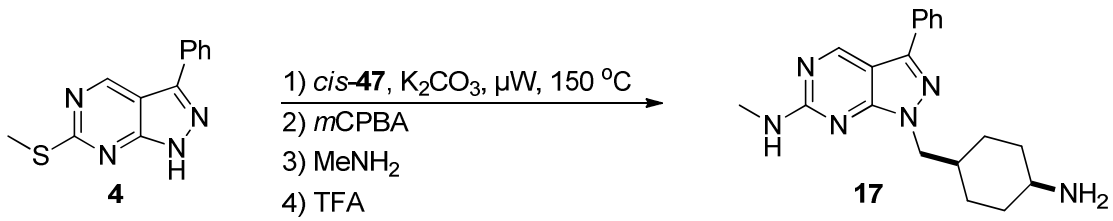
The title compound **15** (0.032 g, 39%) was prepared as a yellow solid according to procedure for **14** from **3** (0.20 g, 0.80 mmol) and *cis*-**50** (0.31 g, 1.0 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 9.01 (s, 1H), 7.92 (d, *J* = 7.7 Hz, 2H), 7.51 (t, *J* = 7.4 Hz, 2H), 7.47–7.39 (t, *J* = 7.7 Hz, 1H), 4.24 (d, *J* = 7.2 Hz, 2H), 3.87 (bs, 1H), 3.03 (s, 3H), 2.17 (bs, 1H), 1.83–1.69 (m, 2H), 1.63-1.40 (m, 6H); LC-MS (ESI+): t_R = 5.527 min, *m/z* 338.2 $[M+1]^+$.



16

1-((*trans*-4-Aminocyclohexyl)methyl)-*N*-methyl-3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-6-amine (16)

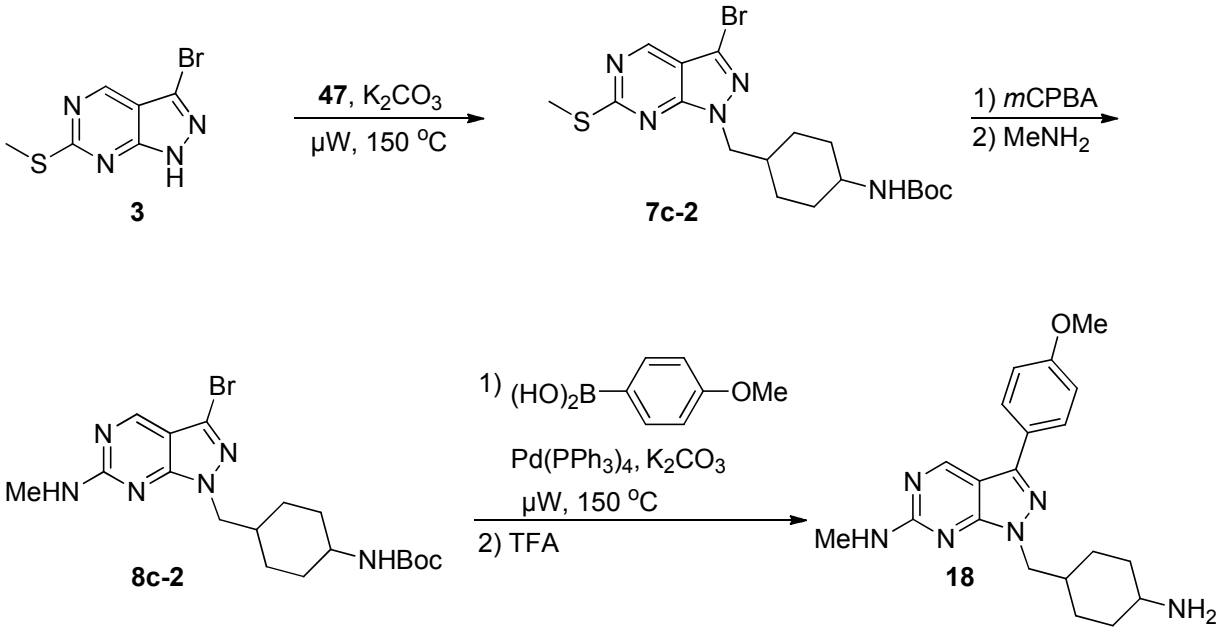
See 'An Example for Path a in Scheme 1'.



1-((*cis*-4-Aminocyclohexyl)methyl)-*N*-methyl-3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-6-amine (17)

The title compound **16** (0.049 g, 52%) was prepared as a white solid according to procedure for **16** from **4** (0.073 g, 0.30 mmol) and *cis*-**47** (0.10 g, 0.36 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 8.95 (s, 1H), 7.90 (d, *J* = 7.3 Hz, 2H), 7.49 (t, *J* = 7.4 Hz, 2H), 7.42 (t, *J* = 7.3 Hz, 1H), 4.31 (d, *J* = 7.6 Hz, 2H), 3.12–3.03 (m, 1H), 3.01 (s, 3H), 2.39–2.24 (m, 1H), 1.82–1.69 (m, 4H), 1.64–1.49 (m, 4H); ^{13}C NMR (100 MHz, CD_3OD) δ 162.9, 157.7, 154.9, 145.8, 133.7, 130.0, 129.9, 128.0, 106.6, 50.1, 49.8, 35.8, 29.7, 28.6, 26.1; LC-MS (ESI+): t_R = 4.000 min, *m/z* 337.3 $[M+1]^+$.

Synthesis of Compounds 18-38 (Table 2): Since the starting material *trans*-4-amino cyclohexanecarboxylic acid was fairly expensive ($73.7/g from TCI), a 1:1 mixture of *trans* and *cis*-isomers was used as starting material instead. The resulting final compounds **18**-**38** are a 2:1 mixture of *cis*:*trans* isomers based on NMR spectra.



***tert*-Butyl 4-((3-bromo-6-(methylthio)-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclohexylcarbamate (7c-2)**

The title compound **7c-2** (0.31 g, 84%) was prepared as a white solid according to general procedure B from **3** (0.20 g, 0.80 mmol) and **47** (0.29 g, 1.0 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (s, *cis*, 0.6H), 8.75 (s, *trans*, 0.4H), 4.62 (bs, *cis*, 0.6H), 4.37 (bs, *trans*, 0.4H), 4.26 (d, *J* = 7.6 Hz, *cis*, 1.2H), 4.20 (d, *J* = 6.8 Hz, *trans*, 0.8H), 3.70 (bs, *cis*, 0.6H), 3.38 (bs, *trans*, 0.4H), 2.62 (s, *cis*, 1.8H), 2.61 (s, *trans*, 1.2H), 2.22–0.99 (series of m, 9H), 1.44 (s, *cis*, 5.4H), 1.41 (s, *trans*, 3.6H); LC-MS (ESI+): t_R(*cis*) = 6.227 min, *m/z* 478.1 $[M+Na]^+$; t_R(*trans*) = 6.165 min, *m/z* 478.1 $[M+Na]^+$.

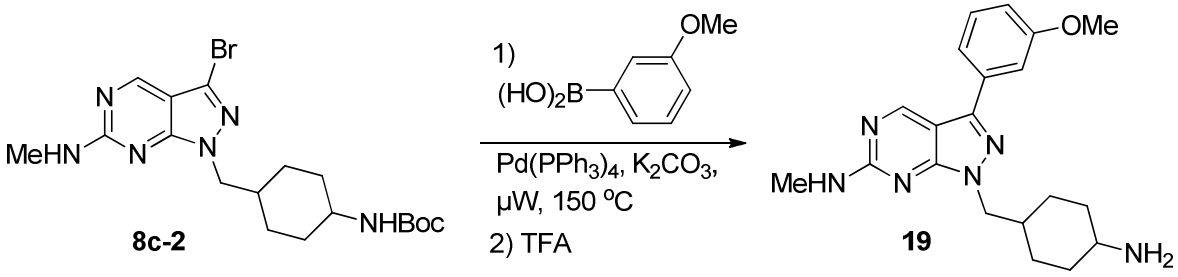
***tert*-Butyl 4-((3-bromo-6-(methylamino)-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclohexylcarbamate (8c-2)**

The title compound **8c-2** (0.41 g, 85%) was prepared as a white solid according general procedure C from **7c-2** (0.46 g, 1.1 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (s, 1H), 6.59 (s, *cis*, 0.66H), 6.47 (s, *trans*, 0.33H), 4.18 (d, *J* = 7.6 Hz, *cis*, 1.32H), 4.09 (d, *J* = 6.8 Hz, *trans*,

0.66H), 3.56 (bs, *cis*, 0.66H), 3.26 (bs, *trans*, 0.33H), 2.99 (s, *cis*, 1.98H), 2.99 (s, *trans*, 0.99H), 2.20–1.08 (series of m, 9H), 1.44 (s, *cis*, 5.94H), 1.42 (s, *trans*, 2.97H); LC-MS (ESI+): t_R(*cis*) = 5.874 min, *m/z* 439.2 $[M+1]^+$; t_R(*trans*) = 5.812 min, *m/z* 439.2 $[M+1]^+$.

1-((4-Aminocyclohexyl)methyl)-3-(4-methoxyphenyl)-*N*-methyl-1*H*-pyrazolo[3,4-d]pyrimidin-6-amine (18)

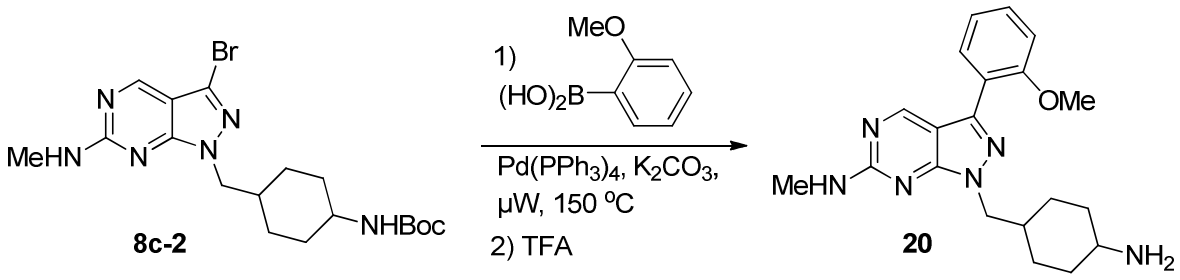
The title compound **18** (0.054 g, 74%) was prepared as a yellow solid accordingtogeneral procedure F from **8c-2** (0.088 g, 0.20 mmol) and 4-methoxyphenylboronic acid (0.046 g, 0.30 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (s, 1H), 7.86 (d, *J* = 8.4 Hz, 2H), 7.07 (d, *J* = 8.4 Hz, 2H), 4.34 (d, *J* = 7.6 Hz, *cis*, 1.32H), 4.21 (d, *J* = 7.2 Hz, *trans*, 0.66H), 3.87 (s, 3H), 3.29–3.23 (m, *cis*, 0.66H), 3.10–3.05 (m, *trans*, 0.33H), 3.03 (s, *cis*, 2H), 3.02 (s, *trans*, 1H), 2.43–1.22 (series of m, 9H); LC-MS (ESI+): t_R(*cis*) = 4.158 min, *m/z* 367.3 $[M+1]^+$; t_R(*trans*) = 4.269 min, *m/z* 367.3 $[M+1]^+$.



1-((4-Aminocyclohexyl)methyl)-3-(3-methoxyphenyl)-*N*-methyl-1*H*-pyrazolo[3,4-d]pyrimidin-6-amine (19)

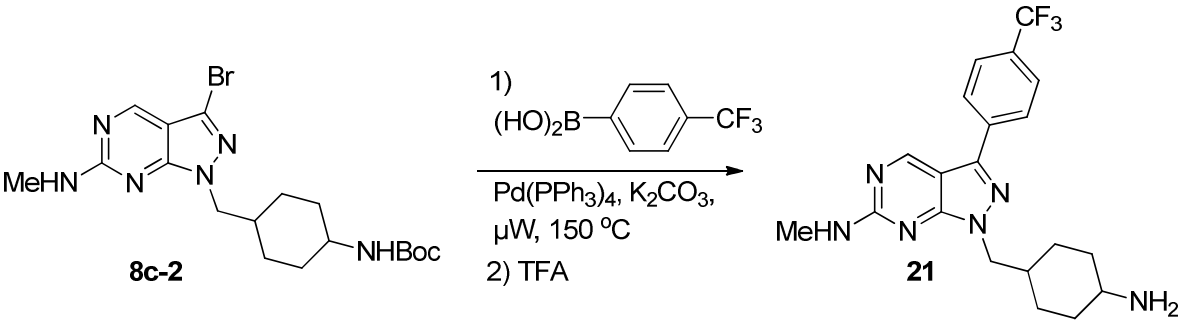
The title compound **19** (0.057 g, 78%) was prepared as a yellow solid according to general procedure F from **8c-2** (0.088 g, 0.20 mmol) and (3-methoxyphenyl)boronic acid (0.046 g, 0.30 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 9.08 (s, 1H), 7.55–7.39 (m, 3H), 7.05 (d, *J* = 7.2 Hz, 1H), 4.37 (d, *J* = 7.6 Hz, *cis*, 1.32H), 4.24 (d, *J* = 6.8 Hz, *trans*, 0.66H), 3.88 (s, *cis*, 1.98 H), 3.88 (s, *trans*, 0.99H), 3.30–3.25 (m, *cis*, 0.66H), 3.15–3.07 (m, *trans*, 0.33H), 3.06 (s, *cis*,

1.98H), 3.05 (s, *trans*, 0.99H), 2.45–1.21 (series of m, 9H); LC-MS (ESI+): t_R(*cis* and *trans*) = 3.973 min, *m/z* 367.2 $[M+1]^+$.



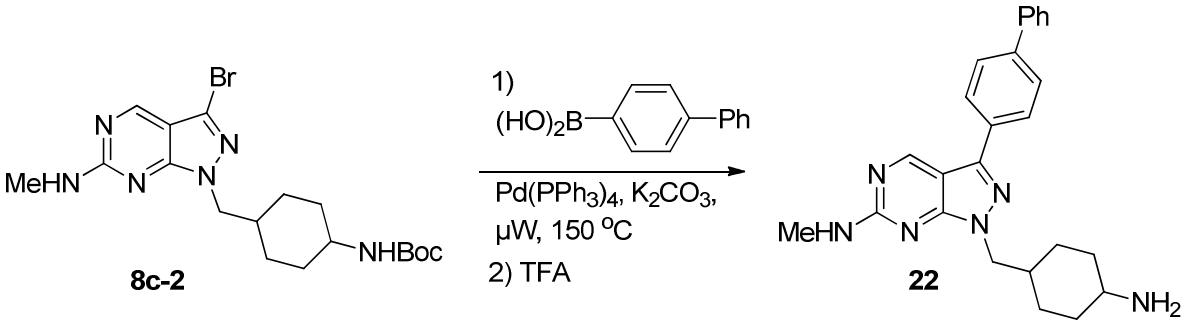
1-((4-Aminocyclohexyl)methyl)-3-(2-methoxyphenyl)-*N*-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (20)

The title compound **20** (0.052 g, 71%) was prepared as a yellow solid according to general procedure F from **8c-2** (0.088 g, 0.20 mmol) and (2-methoxyphenyl)boronic acid (0.046 g, 0.30 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 8.93 (s, 1H), 7.78 (dt, *J* = 7.7, 2.0 Hz, 1H), 7.54–7.45 (m, 1H), 7.20 (d, *J* = 8.3 Hz, 1H), 7.08 (t, *J* = 7.5 Hz, 1H), 4.39 (d, *J* = 7.6 Hz, *cis*, 1.32H), 4.26 (d, *J* = 6.9 Hz, *trans*, 0.66H), 3.94 (s, 3H), 3.36–3.27 (m, *cis*, 0.66H), 3.12–3.03 (m, *trans*, 0.33H), 3.08 (s, *cis*, 1.98H), 3.07 (s, *trans*, 0.99H), 2.45–1.22 (series of m, 9H); LC-MS (ESI+): t_R(*cis* and *trans*) = 3.785 min, *m/z* 367.2 $[M+1]^+$.



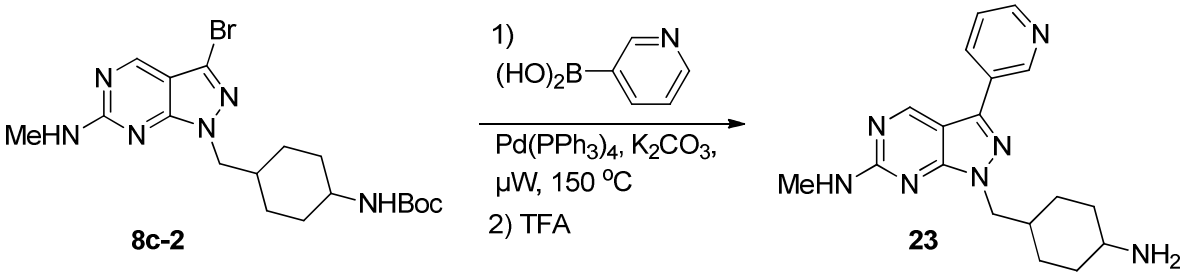
1-((4-Aminocyclohexyl)methyl)-*N*-methyl-3-(4-(trifluoromethyl)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (21)

The title compound **21** (0.032 g, 54%) was prepared as a yellow solid according to general procedure F from **8c-2** (0.050 g, 0.11 mmol) and (4-trifluorphenyl)boronic acid (0.066 g, 0.35 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 9.22 (s, 1H), 8.16 (d, *J* = 8.4 Hz, 2H), 7.83 (d, *J* = 8.4 Hz, 2H), 4.40 (d, *J* = 7.6 Hz, 1.3 H), 4.28 (d, *J* = 6.9 Hz, 0.7 H), 3.31–3.26 (m, *cis*, 0.66H), 3.08 (s, *cis*, 2H), 3.07 (s, *trans*, 1H), 3.08–3.04 (m, *trans*, 0.33H), 2.47–1.23 (series of m, 9H). LC-MS (ESI+): t_R(*cis* and *trans*) = 4.552 min *m/z* 405.2 $[M+1]^+$.



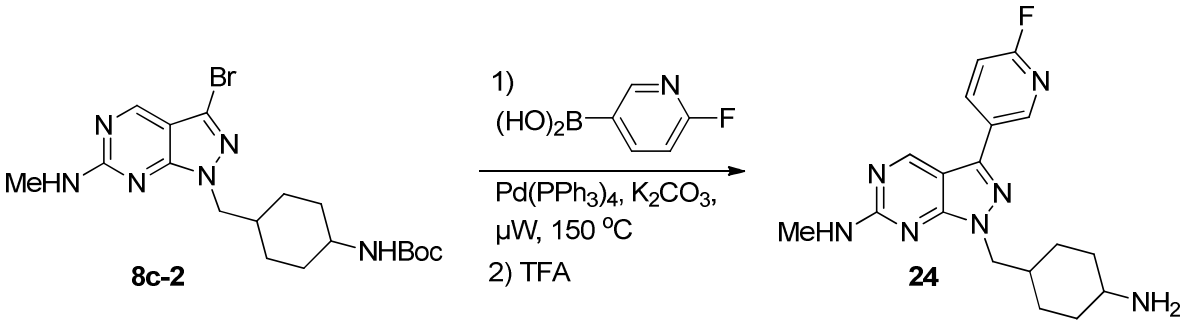
1-((4-Aminocyclohexyl)methyl)-3-(biphenyl-4-yl)-*N*-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (22)

The title compound **22** (0.060 g, 73%) was prepared as a yellow solid according to general procedure F from **8c-2** (0.088 g, 0.20 mmol) and (1,1'-biphenyl)-4-ylboronic acid (g, 0.30 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 9.08 (s, 1H), 8.02 (d, *J* = 8.4 Hz, 2H), 7.78 (d, *J* = 8.4 Hz, 2H), 7.69 (d, *J* = 7.2 Hz, 2H), 7.47 (t, *J* = 7.2 Hz, 2H), 7.38 (t, *J* = 7.2 Hz, 1H), 4.37 (d, *J* = 7.6 Hz, *cis*, 1.32H), 4.25 (d, *J* = 7.2 Hz, *trans*, 0.66H), 3.29–3.24 (m, *cis*, 0.66H), 3.13–3.06 (m, *trans*, 0.33H), 3.04 (s, *cis*, 1.98H), 3.03 (s, *trans*, 0.99H), 2.47–1.23 (series of m, 9H); LC-MS (ESI+): t_R(*cis* and *trans*) = 4.604 min, *m/z* 413.3 $[M+1]^+$.



1-((4-Aminocyclohexyl)methyl)-*N*-methyl-3-(pyridin-3-yl)-1*H*-pyrazolo[3,4-d]pyrimidin-6-amine (23)

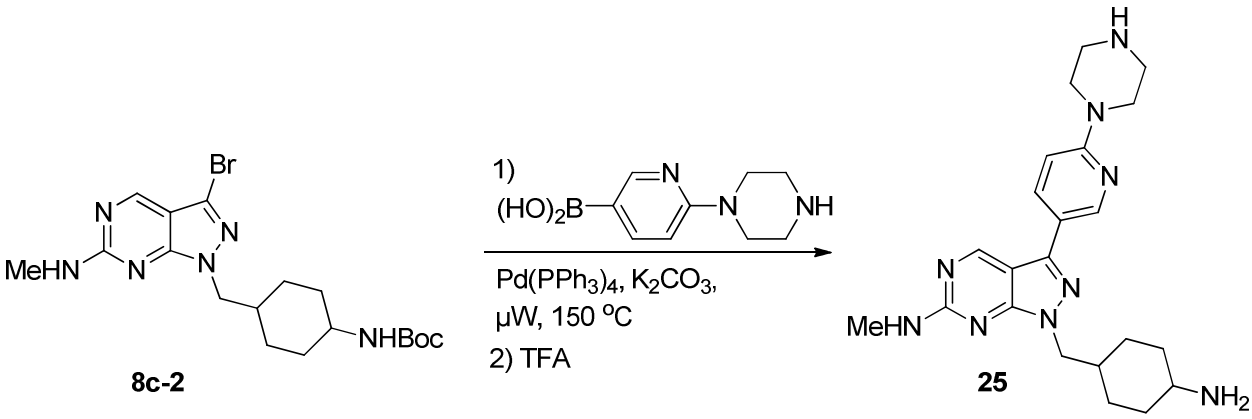
The title compound **23** (0.022 g, 84%) was prepared as a yellow solid according to general procedure F from **8c-2** (0.020 g, 0.046 mmol) and pyridin-3-ylboronic acid (0.014 g, 0.11 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 9.37 (s, 1H), 9.27 (s, 1H), 8.99 (d, *J* = 8.2 Hz, 1H), 8.84 (d, *J* = 5.4 Hz, 1H), 8.07 (dd, *J* = 8.1, 5.6 Hz, 1H), 4.44 (d, *cis*, *J* = 7.6 Hz, 1.31H), 4.31 (d, *trans*, *J* = 6.9 Hz, 0.69 H), 3.31–3.26 (m, *cis*, 0.66H), 3.07 (s, *cis*, 2H), 3.06 (s, *trans*, 1H), 3.08–3.04 (m, *trans*, 0.33H), 2.46–1.22 (series of m, 9H). LC-MS (ESI+): t_R(*cis*) = 3.391 min, *m/z* 338.2 $[M+1]^+$; t_R(*trans*) = 3.594 min, *m/z* 338.3 $[M+1]^+$.



1-((4-Aminocyclohexyl)methyl)-3-(6-fluoropyridin-3-yl)-*N*-methyl-1*H*-pyrazolo[3,4-d]pyrimidin-6-amine (24)

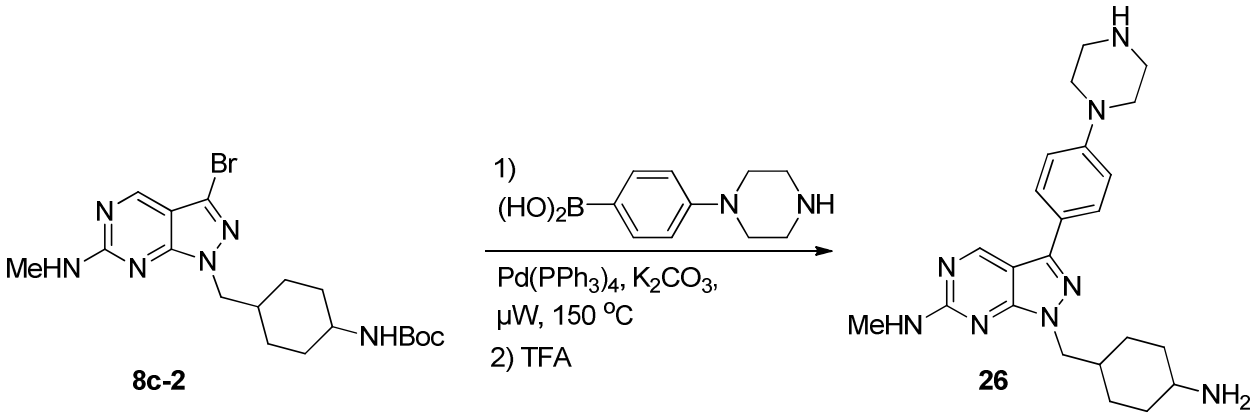
The title compound **24** (0.017 g, 64%) was prepared as a yellow solid according to general procedure F from **8c-2** (0.020g, 0.046 mmol) and (6-fluoropyridin-3-yl)boronic acid (0.019 g, 0.13 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 9.21 (s, 1H), 8.80 (d, *J* = 2.3 Hz, 1H),

8.55–8.48 (m, 1H), 7.25 (dd, *J* = 8.6, 2.5 Hz, 1H), 4.40 (d, *cis*, *J* = 7.6 Hz, 1.3 H), 4.27 (d, *trans*, *J* = 6.9 Hz, 0.7 H), 3.31–3.26 (m, *cis*, 0.66H), 3.084 (s, *cis*, 2H), 3.076 (s, *trans*, 1H), 3.08–3.04 (m, *trans*, 0.33H), 2.45–1.20 (series of m, 9H). LC-MS (ESI+): t_R(*cis*) = 3.694 min, *m/z* 356.2 $[M+1]^+$; t_R(*trans*) = 3.872 min, *m/z* 356.2 $[M+1]^+$.



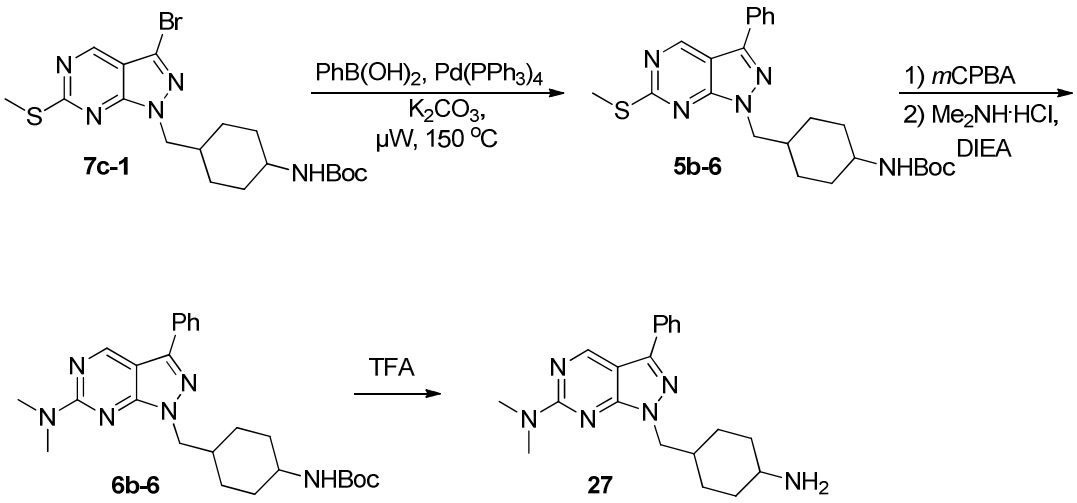
1-((4-Aminocyclohexyl)methyl)-*N*-methyl-3-(6-(piperazin-1-yl)pyridin-3-yl)-1*H*-pyrazolo[3,4-d]pyrimidin-6-amine (25)

The title compound (0.022 g, 54%) was prepared as a yellow solid according to general procedure F from **8c-2** (0.020 g, 0.046 mmol) and (6-(piperazin-1-yl)pyridin-3-yl)boronic acid (0.039 g, 0.13 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 9.20 (s, 1H), 8.76 (d, *J* = 2.2 Hz, 1H), 8.26 (dd, *J* = 9.1, 2.4 Hz, 1H), 7.15 (d, *J* = 9.1 Hz, 1H), 4.38 (d, *cis*, *J* = 7.6 Hz, 1.3H), 4.25 (d, *trans*, *J* = 6.9 Hz, 0.7H), 3.98 (m, 4H), 3.42–3.35 (m, 4H), 3.33–3.29 (m, *cis*, 0.66H), 3.10 (s, *cis*, 2H), 3.09 (s, *trans*, 1H), 3.11–3.06 (m, *trans*, 0.33H), 2.42–1.21 (series of m, 9H). LC-MS (ESI+): t_R(*cis*) = 2.618 min, *m/z* 422.3 $[M+1]^+$; t_R(*trans*) = 2.899 min, *m/z* 422.3 $[M+1]^+$.



1-((4-Aminocyclohexyl)methyl)-*N*-methyl-3-(4-(piperazin-1-yl)phenyl)-1*H*-pyrazolo[3,4-d]pyrimidin-6-amine (26)

The title compound **26** (0.068 g, 81%) was prepared as a yellow solid according to general procedure F from **8c-2** (0.088 g, 0.20 mmol) and (4-(piperazin-1-yl)phenyl)boronic acid (0.21 g, 1.0 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 9.17 (s, *cis*, 0.66H), 9.16 (s, *trans*, 0.33H), 7.88 (m, 2H), 7.14 (d, J = 8.8 Hz, 2H), 3.56 (m, 4H), 3.40 (m, 4H), 3.31–3.26 (m, *cis*, 0.66H), 3.10 (s, *cis*, 1.98H), 3.09 (s, *trans*, 0.99H), 3.08–3.04 (m, *trans*, 0.33H), 2.44–1.21 (series of m, 9H); LC-MS (ESI+): t_R(*cis*) = 2.756 min, *m/z* 421.3 $[M+1]^+$; t_R(*trans*) = 2.982 min, *m/z* 421.3 $[M+1]^+$.



***tert*-Butyl 4-((6-(methylthio)-3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclohexylcarbamate (5b-6)**

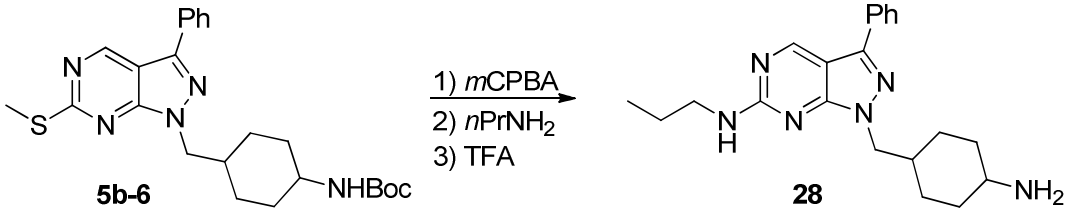
The title compound **5b-6** (0.080 g, 88%) was prepared as a white solid according to general procedure A from **7c-1** (0.092 g, 0.20 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (s, *cis*, 0.66H), 9.14 (s, *trans*, 0.33H), 7.97- 7.89 (dt, *J* = 6.8, 1.6 Hz, 2H), 7.56–7.48 (tt , *J* = 7.2, 1.6 Hz, 2H), 7.47–7.40 (tt, *J* = 7.2, 1.6 Hz, 1H), 4.35 (d, *J* = 7.2 Hz, *cis*, 1.32H), 4.29 (d, *J* = 7.2 Hz, *trans*, 0.66H), 3.72 (bs, *cis*, 0.66H), 3.40 (bs, *trans*, 0.33H), 2.66 (s, *cis*, 1.98H), 2.65 (s, *trans*, 0.99H), 2.30–1.00 (series of m, 9H), 1.45 (s, *cis*, 5.94H), 1.42 (s, *trans*, 2.97H); LC-MS (ESI+): t_R(*cis*) = 6.388 min, *m/z* 454.3 $[M+1]^+$; t_R(*trans*) = 6.452 min, *m/z* 454.3 $[M+1]^+$.

***tert*-Butyl 4-((6-(dimethylamino)-3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclohexylcarbamate (6b-6)**

The title compound **6b-6** (0.067 g, 84%) was prepared as a white solid according to general procedure C from **5b-6** (0.080 g, 0.17 mmol) using 10 eq dimethylamine hydrochloride and 10 eq *N*,*N*-diisopropylethylamine for 16 h at 60 °C.

***tert*-Butyl 4-((6-(dimethylamino)-3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclohexylcarbamate (27)**

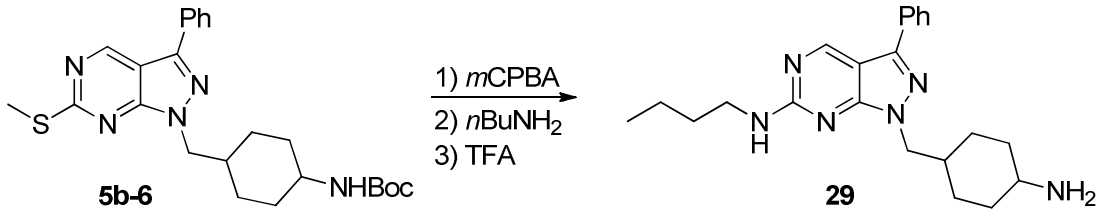
The title compound **27** (0.046 g, 89%) was prepared as a yellow solid according to general procedure D from **6b-6** (0.067 g, 0.15 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 9.022 (s, *trans*, 0.33H), 9.017 (s, *cis*, 0.66H), 7.90 (d, *J* = 3.6 Hz, 2H), 7.49 (t, *J* = 7.2 Hz, 2H), 7.43 (t, *J* = 7.2 Hz, 1H), 4.31 (d, *J* = 7.6 Hz, *cis*, 1.32H), 4.18 (d, *J* = 6.8 Hz, *trans*, 0.66H), 3.31–3.29 (m, 0.66H), 3.27 (s, *cis*, 1.98H), 3.26 (s, *trans*, 0.99H), 3.10–3.01 (m, 0.33H), 2.42–1.18 (series of m, 9H); LC-MS (ESI+): t_R(*cis*) = 4.521 min, *m/z* 351.3 $[M+1]^+$; t_R(*trans*) = 4.625 min, *m/z* 351.3 $[M+1]^+$.



5b-6 **28**

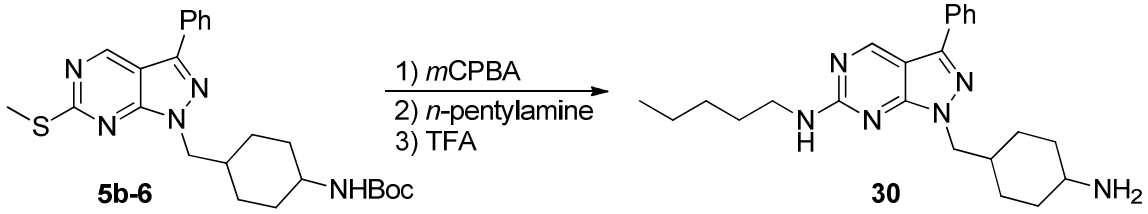
***tert*-Butyl 4-((3-phenyl-6-(propylamino)-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclohexylcarbamate (28)**

The title compound **28** (0.054 g, 74%) was prepared as a yellow solid according to general procedure C and D from **5b-6** (0.091 g, 0.20 mmol) using *n*-propylamine (0.059 g, 1.0 mmol) for 2.0 h at 60 °C**.** ^{1}H NMR (400 MHz, CD_3OD) δ 9.11 (s, *cis*, 0.66H), 9.10 (s, *trans*, 0.33H), 7.93 (d, *J* = 7.6 Hz, 2H), 7.58–7.42 (m, 3H), 4.35 (d, *J* = 7.2 Hz, *cis*, 1.32H), 4.23 (d, *J* = 6.4 Hz, *trans*, 0.66H), 3.55–3.43 (m, 2H), 3.34–3.31 (m, *cis*, 0.66H), 3.11–3.02 (m, *trans*, 0.33H), 2.43–1.22 (series of m, 11H), 1.07–0.98 (m, 3H); LC-MS (ESI+): t_R(*cis*) = 4.488 min, *m/z* 365.3 $[M+1]^+$; t_R(*trans*) = 4.561 min, *m/z* 365.3 $[M+1]^+$.



***tert*-Butyl 4-((6-(butylamino)-3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclohexylcarbamate (29)**

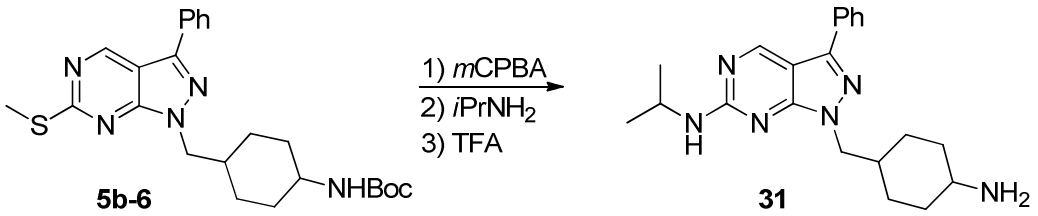
The title compound **29** (0.054 g, 71%) was prepared as a yellow solid according to general procedure C and D from **5b-6** (0.091 g, 0.20 mmol) using *n*-butylamine (0.073 g, 1.0 mmol) for 2.0 h at 60 °C**.** ^{1}H NMR (400 MHz, CD_3OD) δ 8.96 (s, 1H), 7.90 (t, *J* = 7.2 Hz, 2H), 7.42 (t, *J* = 7.2 Hz, 2H), 4.31 (d, *J* = 7.2 Hz, *cis*, 1.32 H), 4.18 (d, *J* = 6.8 Hz, *trans*, 0.66H), 3.53–3.40 (m, 2H), 3.30–3.23 (m, *cis*, 0.66H), 3.10–2.97 (m, *trans*, 0.33H), 2.41–1.18 (series of m, 13H), 0.99 (t, *J* = 7.2 Hz, 3H); LC-MS (ESI+): t_R(*cis*) = 4.686 min, *m/z* 379.3 $[M+1]^+$; t_R(*trans*) = 4.797 min, *m/z* 379.3 $[M+1]^+$.



***tert*-Butyl 4-((6-(pentylamino)-3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclohexylcarbamate (30)**

The title compound **30** (0.058 g, 74%) was prepared as a yellow solid according to general procedure C and D from **5b-6** (0.091 g, 0.20 mmol) using *n*-pentylamine (0.087 g, 1.0 mmol) for 2.0 h at 60 °C**.** ^{1}H NMR (400 MHz, CD_3OD) δ 9.02 (s, *cis*, 0.66H), 9.01 (s, *trans*, 0.33H), 7.94–7.88 (m, 2H), 7.54–7.47 (m, 2H), 7.47 - 7.40 (m, 1H), 4.31 (d, *J* = 7.6 Hz, *cis*,

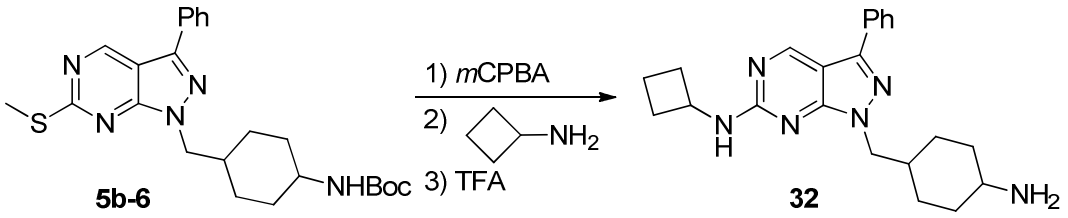
1.32H), 4.19 (d, *J* =7.2 Hz, *trans*, 0.66H), 3.48 (t, *J* = 6.8 Hz, *cis*, 1.32H), 3.47 (t, *J* = 0.66 Hz, *trans*, 0.66H), 3.35–3.27 (m, *cis*, 0.66H), 3.10–3.00 (m, *trans*, 0.33H), 2.42–1.20 (series of m, 15H), 1.02–0.90 (m, 3H); LC-MS (ESI+): t_R(*cis* and *trans*) = 4.878 min, *m/z* 393.3 $[M+1]^+$.



5b-6 **31**

***tert*-Butyl 4-((6-(isopropylamino)-3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclohexylcarbamate (31)**

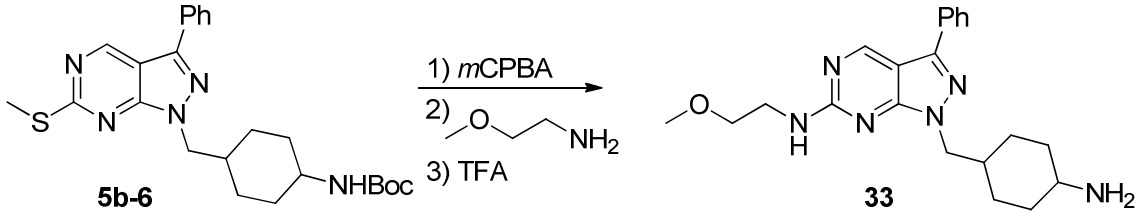
The title compound **31** (0.047 g, 65%) was prepared as a yellow solid according to general procedure C and D from **5b-6** (0.091 g, 0.20 mmol) using isopropylamine (0.059 g, 1.0 mmol) for 6.0 h at 60 °C**.** ^{1}H NMR (400 MHz, CD_3OD) δ 9.11 (s, 1H), 7.93 (d, *J* = 8.0 Hz, 2H), 7.58–7.40 (m, 3H), 4.35 (d, *J* = 7.6 Hz, *cis*, 1.32H), 4.34–4.24 (m, 1H), 4.23 (d, *J* = 7.2 Hz, *trans*, 0.66H), 3.37–3.27 (m, 0.66H), 3.13–3.01 (m, 0.33H), 2.44–1.21 (series of m, 9H), 1.32 (d, *J* = 6.8 Hz, 6H); LC-MS (ESI+): t_R(*cis*) = 4.435 min, *m/z* 365.3 $[M+1]^+$; t_R(*trans*) = 4.565 min, *m/z* 365.3 $[M+1]^+$.



5b-6 **32**

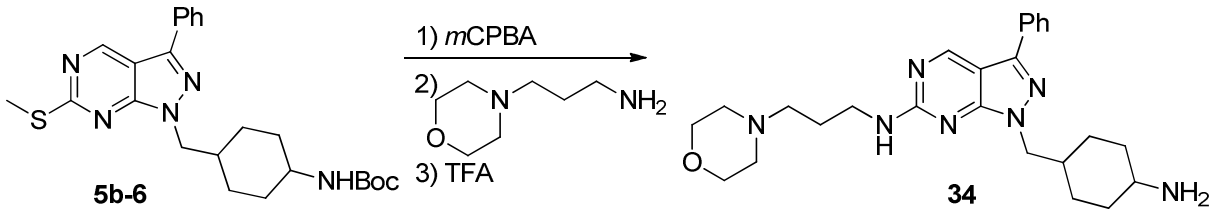
***tert*-Butyl 4-((6-(cyclobutylamino)-3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclohexylcarbamate (32)**

The title compound **32** (0.057g, 76%) was prepared as a yellow solid according to general procedure C and D from **5b-6** (0.091 g, 0.20 mmol) using cyclobutylamine (0.071 g, 1.0 mmol)) for 6.0 h at 60 °C**.** ^{1}H NMR (400 MHz, CD_3OD) δ 9.00 (s, 1H), 7.94–7.85 (m, 2H), 7.52–7.46 (m, 2H), 7.46–7.40 (m, 1H), 4.61–4.44 (m, 1H), 2.30 (d, *J* = 7.6 Hz, *cis*, 1.32H), 4.18 (d, *J* = 6.8 Hz, *trans*, 0.66H), 3.34–3.26 (m, *cis*, 0.66H), 3.13–3.00 (m, *trans*, 0.33H), 2.50–1.19 (series of m, 15H); LC-MS (ESI+): t_R(*cis*) = 4.517 min, *m/z* 377.3 $[M+1]^+$; t_R(*trans*) = 4.648 min, *m/z* 377.3 $[M+1]^+$.



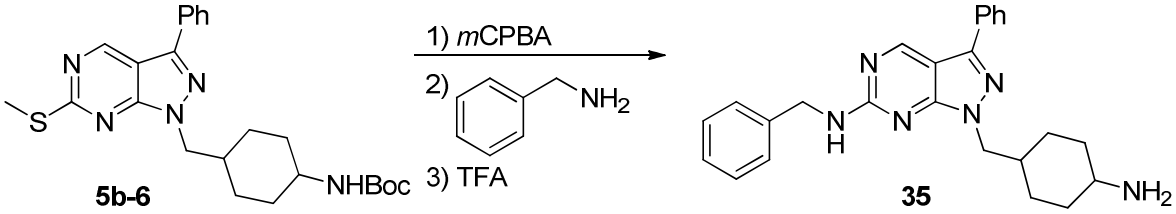
***tert*-Butyl 4-((6-(2-methoxyethylamino)-3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclohexylcarbamate (33)**

The title compound **33** (0.054 g, 71%) was prepared as a yellow solid according to general procedure C and D from **5b-6** (0.091 g, 0.20 mmol) using 2-methoxyethanamine (0.075 g, 1.0 mmol) for 2.0 h at 60 °C**.** ^{1}H NMR (400 MHz, CD_3OD) δ 9.09 (s, 1H), 7.97–7.89 (m, 2H), 7.56–7.49 (m, 2H), 7.49–7.43 (m, 1H), 4.34 (d, *J* = 7.6 Hz, *cis*, 1.32H), 4.22 (d, *J* = 6.8 Hz, *trans*, 0.66H), 3.74–3.67 (m, 2H), 3.67–3.60 (m, 2H), 3.41 (s, 3H), 3.34–3.26 (m, *cis*, 0.66H), 3.12–3.01 (m, *trans*, 0.33H), 2.43–1.20 (series of m, 9H; LC-MS (ESI+): t_R(*cis* and *trans*) = 4.217 min, *m/z* 381.3 $[M+1]^+$.



***tert*-Butyl 4-((6-(3-morpholinopropylamino)-3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclohexylcarbamate (34)**

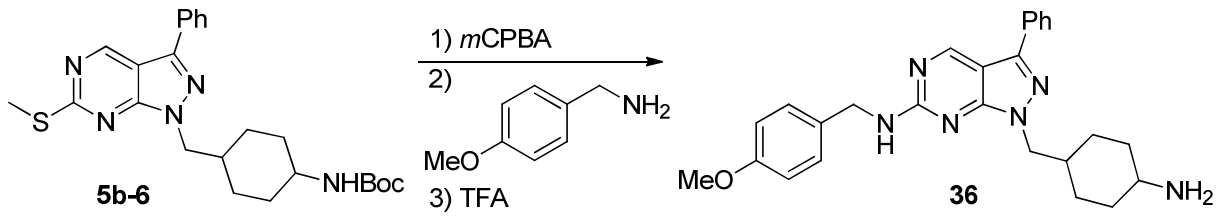
The title compound **34** (0.062 g, 69%) was prepared as a yellow solid according to general procedure C and D from **5b-6** (0.091 g, 0.20 mmol) using 3-morpholinopropan-1-amine (0.14 g, 1.0 mmol) for 2.0 h at 60 °C**.** ^{1}H NMR (400 MHz, CD_3OD) δ 9.16 (s, 1H), 7.99–7.89 (m, 2H), 7.58–7.43 (m, 3H), 4.35 (d, *J* = 7.6 Hz, *cis*, 1.32H), 4.23 (d, *J* = 6.8 Hz, *trans*, 0.66H), 4.12–3.98 (m, 2H), 3.89–3.73 (m, 2H), 3.71–3.62 (m, 2H), 3.60–3.44 (m, 2H), 3.39–3.31 (m, 2H), 3.31- 3.27 (m, *cis*, 0.66H), 3.23–3.10 (m, 2H), 3.10–3.02 (m, *trans*, 0.33H), 2.43–1.22 (series of m, 11H); LC-MS (ESI+): t_R(*cis* and *trans*) = 3.058 min, *m/z* 450.3 $[M+1]^+$.



***tert*-Butyl 4-((6-(benzylamino)-3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclohexylcarbamate (35)**

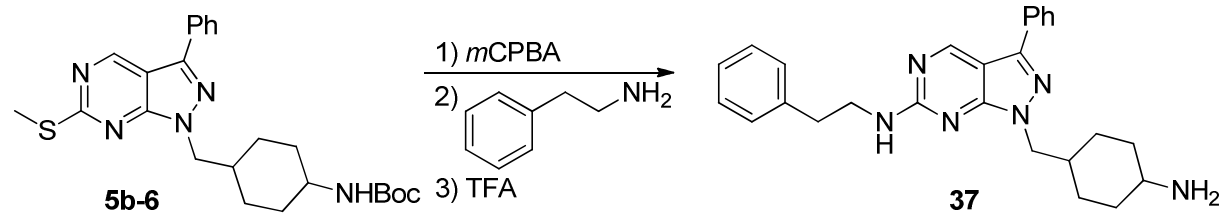
The title compound **35** (0.069 g, 84%) was prepared as a yellow solid according to general procedure C and D from **5b-6** (0.091 g, 0.20 mmol) using benzylamine (0.11 g, 1.0 mmol) for 8.0 h at 60 °C**.** ^{1}H NMR (400 MHz, CD_3OD) δ 9.053 (s, *cis*, 0.66H), 9.049 (s, *trans*, 0.33H), 7.95–7.87 (m, 2H), 7.51 (t, J = 8.0 Hz, 2H), 7.47–7.43 (m, 1H), 7.43–7.37 (m, 2H), 7.32 (t, *J* = 8.0 Hz, 2H), 7.28–7.21 (m, 1H), 4.71 (s, *cis*, 1.32H), 4.67 (s, *trans*, 0.66H), 4.30 (d, *J* =

7.6 Hz, *cis*, 1.32H), 4.15 (d, *J* = 7.2 Hz, *trans*, 0.66H), 3.30–3.23 (m, *cis*, 0.66H), 3.04–2.93 (m, *trans*, 0.33H), 2.32–1.02 (series of m, 9H); LC-MS (ESI+): t_R(*cis* and *trans*) = 4.765 min, *m/z* 413.3 $[M+1]^+$.



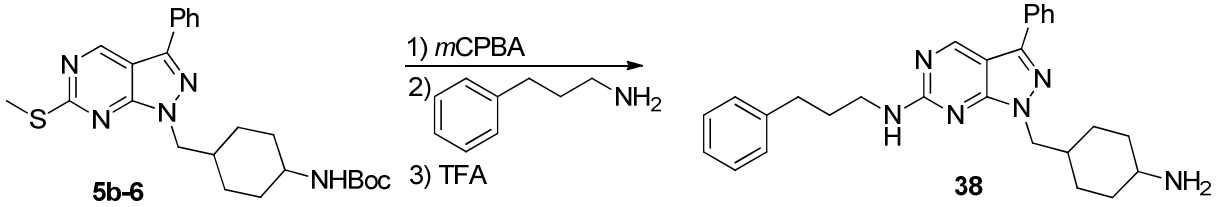
***tert*-Butyl 4-((6-(4-methoxybenzylamino)-3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclohexylcarbamate (36)**

The title compound **36** (0.031 g, 48%) was prepared as a yellow solid according to general procedure C and D from **5b-6** (0.053 g, 0.12 mmol) using 4-methoxybenzylamine (0.19 g, 1.4 mmol) for 8.0 h at 60 °C**.** ^{1}H NMR (400 MHz, CD_3OD) δ 9.13 (s, 1H), 7.93 (d, *J* = 7.4 Hz, 2H), 7.58–7.42 (m, 3H), 7.35 (dd, *J* = 8.2, 5.3 Hz, 2H), 6.89 (d, *J* = 8.5 Hz, 2H), 4.65 (s, *cis*, 1.3H), 4.62 (s, *trans*, 0.7H), 4.34 (d, *cis*, *J* = 7.4 Hz, 1.3H), 4.20 (d, *trans*, *J* = 6.8 Hz, 0.7H), 3.77 (s, 3H), 3.35–3.26 (m, *cis*, 0.66H), 3.07–2.97 (m, *trans*, 0.33H), 2.35–1.09 (series of m, 9H). LC-MS (ESI+): t_R(*cis* and *trans*) = 4.417 min *m/z* 443.3 $[M+1]^+$.



***tert*-Butyl 4-((6-(phenethylamino)-3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclohexylcarbamate (37)**

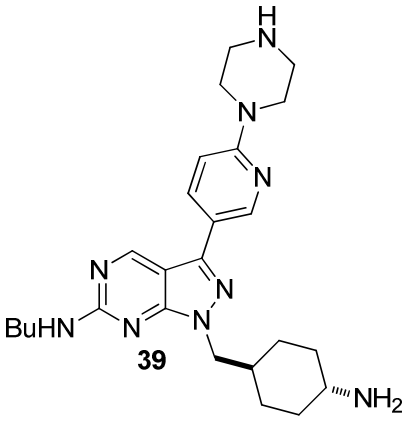
The title compound **37** (0.073 g, 85%) was prepared as a yellow solid according to general procedure C and D from **5b-6** (0.091 g, 0.20 mmol) using 2-phenylethanamine (0.12 g, 1.0 mmol) for 8.0 h at 60 °C**.** ^{1}H NMR (400 MHz, CD_3OD) δ 9.02 (s, 1H), 7.96–7.86 (m, 2H), 7.55–7.47 (m, 2H), 7,47–7.41 (m, 1H), 7.34–7.25 (m, 4H), 7.24–7.15 (m, 1H), 4.32 (d, *J* = 7.6 Hz, *cis*, 1.32H), 4.21 (d, *J* = 7.2 Hz, *trans*, 0.66H), 3.74 (t, *J* = 7.2 Hz, *cis*, 1.32H), 3.71 (t, *J* = 7.6 Hz, *trans*, 0.66H), 3.31–3.27 (m, *cis*, 0.66H), 3.10–3.02 (m, *trans*, 0.33H), 2.98 (t, *J* = 7.2 Hz, 2H), 2.42–1.20 (series of m, 9H); LC-MS (ESI+): t_R(*cis* and *trans*) = 4.826 min, *m/z* 427.3 $[M+1]^+$.



***tert*-Butyl 4-((3-phenyl-6-(3-phenylpropylamino)-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclohexylcarbamate (38)**

The title compound **38** (0.023 g, 35%) was prepared as a yellow solid according to general procedure C and D from **5b-6** (0.053 g, 0.12 mmol) using 3-phenylpropan-1-amine (0.10 g, 0.57 mmol) for 8.0 h at 60 °C**.** ^{1}H NMR (400 MHz, CD_3OD) δ 9.11 (s, 1H), 7.97–7.92 (m, 2H), 7.58–7.42 (m, 3H), 7.32–7.12 (m, 5H), 4.33 (d, *cis*, *J* = 7.4 Hz, 1.3H), 4.19 (d, *trans*, *J* = 6.7 Hz, 0.7H), 3.60–3.50 (m, 2H), 3.35–3.26 (m, *cis*, 0.6H), 3.11–3.06 (m, *trans*, 0.4H), 2.76 (t, *J* = 7.6 Hz, 2H), 2.39–1.17 (series of m, 11H). LC-MS (ESI+): t_R(*cis* and *trans*) = 4.976 min *m/z* 441.3 $[M+1]^+$.

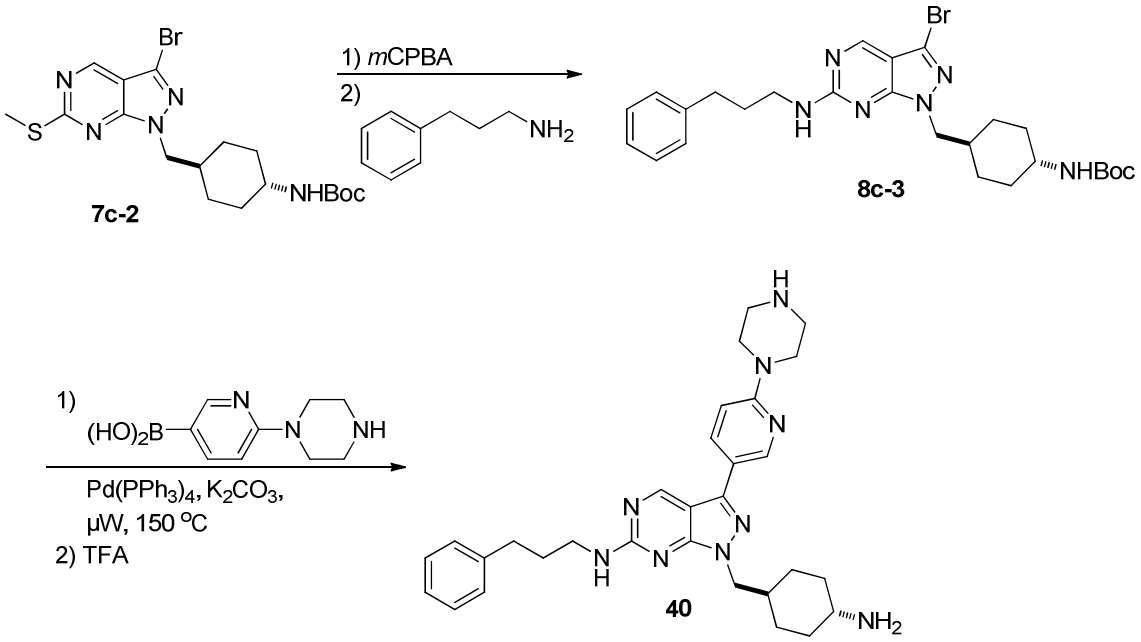
Synthesis of Compounds 39-44 (Table 3):



39

1-((*trans*-4-Aminocyclohexyl)methyl)-*N*-butyl-3-(6-(piperazin-1-yl)pyridin-3-yl)-1*H*-pyrazolo[3,4-d]pyrimidin-6-amine (39)

See 'An Example for Path c in Scheme 1'.



7c-2 8c-3 40

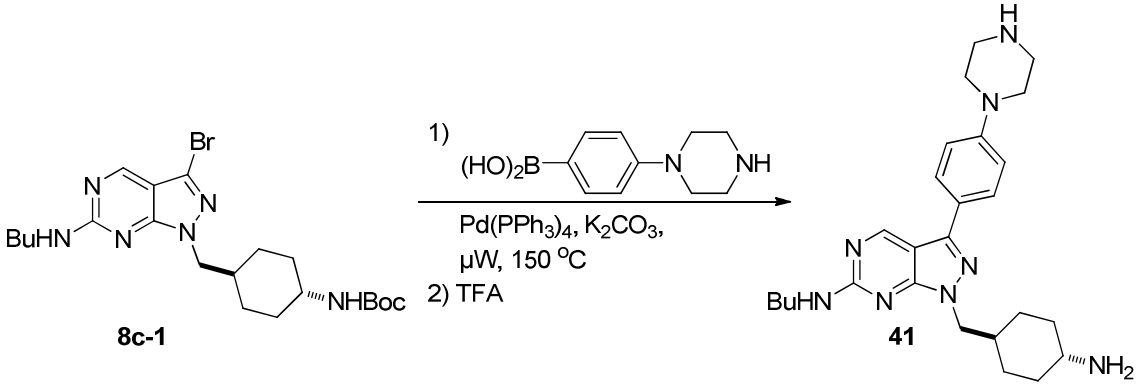
***tert*-Butyl *trans*-4-((3-bromo-6-(3-phenylpropylamino)-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclohexylcarbamate (8c-3)**

The title compound **8c-3** (0.43 g, 73%) was prepared as a white solid according to general procedure C from 7c-2 (0.50 g, 1.08 mmol) and 3-phenylpropan-1-amine (0.50 g, 3.7

mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (s, 1H), 7.35–7.14 (m, 5H), 5.43 (bs, 1H), 4.33 (bs, 1H), 4.04 (d, *J* = 7.0 Hz, 2H), 3.50 (dd, *J* = 12.8, 6.3 Hz, 2H), 3.39 (bs, 1H), 2.74 (t, *J* = 7.6 Hz, 2H), 2.06–1.89 (m, 5H), 1.76–1.59 (m, 2H), 1.43 (s, 9H), 1.24–0.93 (m, 4H).

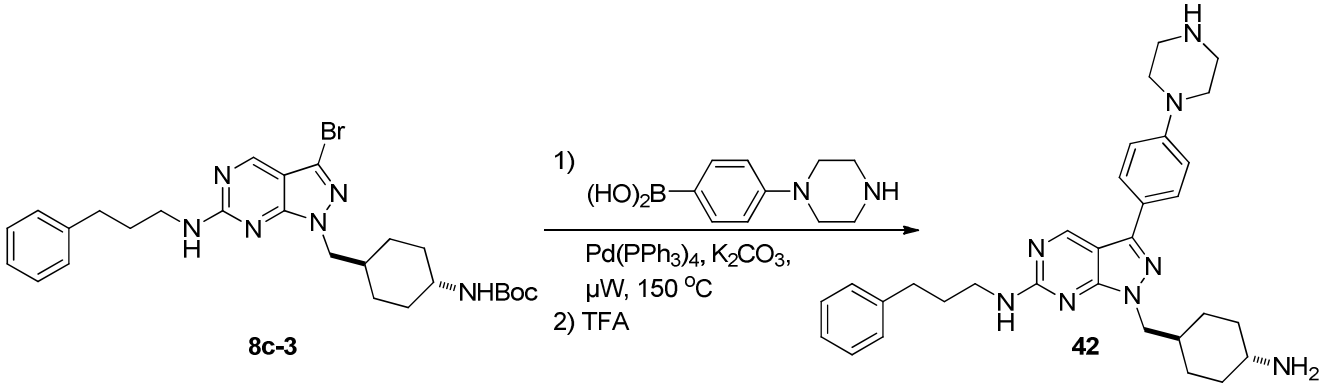
1-((*trans*-4-Aminocyclohexyl)methyl)-*N*-(3-phenylpropyl)-3-(6-(piperazin-1-yl)pyridin-3-yl)-1*H*-pyrazolo[3,4-d]pyrimidin-6-amine (40)

The title compound **40** (0.094 g, 58%) was prepared as a yellow solid according to general procedure F from **8c-3** (0.090 g, 0.17 mmol) and 6-(piperazin-1-yl)pyridin-3-ylboronic acid (0.070 g, 0.24 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 9.14 (s, 1H), 8.76 (d, *J* = 2.2 Hz, 1H), 8.22 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.31–7.21 (m, 4H), 7.21–7.15 (m, 1H), 7.10 (d, *J* = 9.0 Hz, 1H), 4.18 (d, *J* = 6.7 Hz, 2H), 3.99–3.91 (m, 4H), 3.55 (t, *J* = 7.1 Hz, 2H), 3.41–3.33 (m, 4H), 3.09–2.98 (m, 1H), 2.76 (t, *J* = 7.5 Hz, 2H), 2.11–1.97 (m, 5H), 1.82 (d, *J* = 11.7 Hz, 2H), 1.38 (dd, *J* = 23.7, 11.0 Hz, 2H), 1.23 (dd, *J* = 25.0, 11.1 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 162.6, 159.6, 157.2, 149.6, 146.9, 145.3, 143.0, 137.8, 129.5, 129.4, 127.0, 119.4, 109.2, 106.6, 52.6, 51.2, 44.3, 43.3, 42.1, 38.1, 34.1, 31.5, 31.2, 29.5; LC-MS (ESI+): t_R = 3.967 min, *m/z* 525.4 $[M+1]^+$; HRMS (TOF, ESI) *m/z*: $[M+H]^+$ calculated for $C_{30}H_{40}N_9$: 526.3407, found: 526.3440; HPLC: t_R = 7.530 min; purity: 96%.



1-((*Trans*-4-aminocyclohexyl)methyl)-*N*-butyl-3-(4-(piperazin-1-yl)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (41)

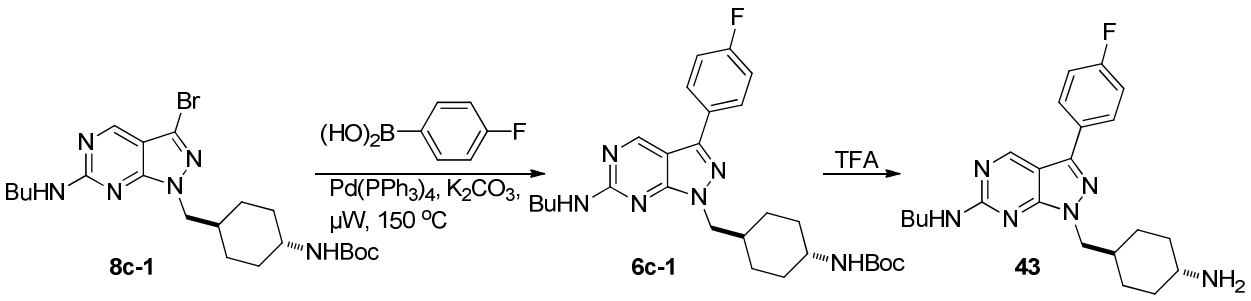
The title compound **41** (0.117 g, 90%) was prepared as a yellow solid according to general procedure F from **8c-1** (0.090 g, 0.19 mmol) and 4-(piperazin-1-yl)phenylboronic acid (0.086 g, 0.28 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 9.14 (s, 1H), 7.90 (d, *J* = 8.7 Hz, 2H), 7.17 (d, *J* = 8.8 Hz, 2H), 4.22 (d, *J* = 6.8 Hz, 2H), 3.60 - 3.50 (m, 6H), 3.44–3.37 (m, 4H), 3.15–3.07 (m, 1H), 2.14–2.01 (m, 3H), 1.87 (d, *J* = 11.9 Hz, 2H), 1.75–1.62 (m, 2H), 1.54–1.20 (m, 6H), 1.01 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 161.0, 155.2, 150.5, 147.8, 145.6, 127.2, 122.4, 115.5, 104.8, 50.6, 49.2, 47.0, 44.9, 42.6, 40.2, 30.1, 29.2, 27.6, 19.1, 12.2; LC-MS (ESI+): t_R = 3.698 min, *m/z* 463.35 $[M+1]^+$; HRMS (TOF, ESI) *m/z*: $[M+H]^+$ calculated for $C_{26}H_{39}N_8$: 463.3298, found: 463.3315; HPLC: t_R = 6.957 min; purity: 95%.



1-((*trans*-4-Aminocyclohexyl)methyl)-*N*-(3-phenylpropyl)-3-(4-(piperazin-1-yl)phenyl)-1*H*-pyrazolo[3,4-d]pyrimidin-6-amine (42)

The title compound **42** (0.080 g, 59%) was prepared as a yellow solid according to general procedure F from **8c-3** (0.085 g, 0.10 mmol) and 4-(piperazin-1-yl)phenylboronic acid (0.072 g, 0.23 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 9.08 (s, 1H), 7.89 (d, *J* = 8.9 Hz, 2H), 7.31–7.21 (m, 4H), 7.21–7.14 (m, 3H), 4.17 (d, *J* = 6.8 Hz, 2H), 3.59–3.50 (m, 6H), 3.44–3.37 (m, 4H), 3.09–2.97 (m, 1H), 2.76 (t, *J* = 7.5 Hz, 2H), 2.11–1.95 (m, 5H), 1.82 (d, *J* = 11.9 Hz, 2H), 1.37 (dd, *J* = 23.8, 11.5 Hz, 2H), 1.23 (dd, *J* = 24.7, 11.3 Hz, 2H); ^{13}C NMR (100 MHz,

CD_3OD) δ 160.6, 155.1, 150.7, 146.3, 146.3, 140.9, 127.5, 127.4, 127.2, 125.0, 122.0, 115.5, 105.0, 50.6, 49.2, 47.0, 44.8, 42.6, 40.1, 36.1, 32.1, 29.4, 29.2, 27.5; LC-MS (ESI+): t_R = 4.444 min, *m/z* 525.4 $[M+1]^+$; HRMS (TOF, ESI) *m/z*: $[M+H]^+$ calculated for $C_{31}H_{41}N_8$: 525.3454, found: 525.3456; HPLC: t_R = 7.543 min; purity: 98%.



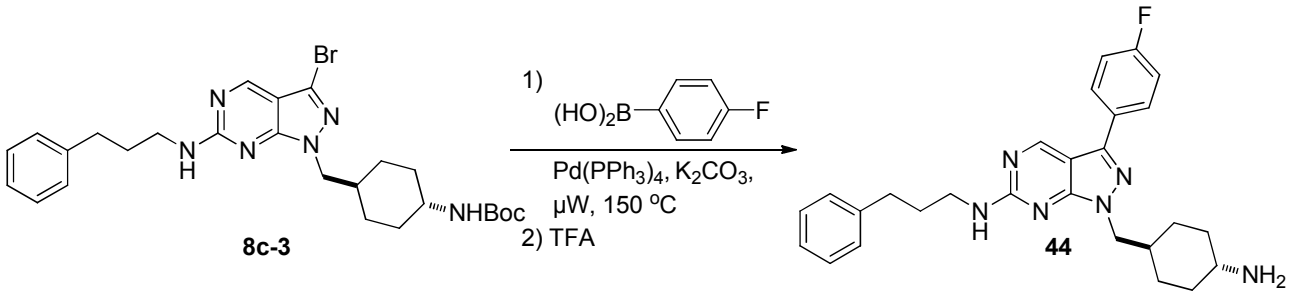
***tert*-Butyl *trans*-4-((6-(butylamino)-3-(4-fluorophenyl)-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)cyclohexylcarbamate (6c-1)**

The title compound **6c-1** (0.069 g, 69%) was prepared as a white solid according to general procedure A from **8c-1** (0.085 g, 0.19 mmol) and 4-fluorophenylboronic acid (0.042 g, 0.30 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (s, 1H), 7.87–7.78 (m, 2H), 7.19–7.11 (m, 2H), 4.48 (bs, 1H), 4.13 (d, *J* = 7.1 Hz, 2H), 3.46 (dd, *J* = 12.6, 6.8 Hz, 2H), 3.39 (bs, 1H), 2.03–1.92 (m, 3H), 1.75–1.58 (m, 4H), 1.42 (s, 9H), 1.28–1.00 (m, 5H), 0.96 (t, *J* = 7.3 Hz, 3H). LC-MS (ESI+): t_R = 6.489 min, *m/z* 497.3 $[M+1]^+$.

1-((*trans*-4-Aminocyclohexyl)methyl)-*N*-butyl-3-(4-fluorophenyl)-1*H*-pyrazolo[3,4-d]pyrimidin-6-amine (43)

The title compound **43** (62 mg, 88%) was prepared as a white solid according general procedure D from **6c-1** (0.069g, 0.13 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 9.08 (s, 1H), 8.02–7.92 (m, 2H), 7.33-7.20 (m, 2H), 4.22 (m, 2H), 3.51 (t, *J* = 7.1 Hz, 2H), 3.13–3.00 (m, 1H), 2.15–1.98 (m, 3H), 1.85 (d, *J* = 12.2 Hz, 2H), 1.74–1.62 (m, 2H), 1.55–1.20 (m, 6H), 1.00 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 164.6 ($^1J_{CF}$ = 246 Hz), 162.3, 157.7, 154.9, 144.7,

130.1 ($^4J_{CF}$ = 3.2 Hz), 129.9 ($^3J_{CF}$ = 8.3 Hz), 116.7 ($^2J_{CF}$ = 21.9 Hz), 106.5, 52.2, 51.3, 42.1, 38.4, 32.6, 31.3, 29.6, 21.2, 14.3, 52.2, 51.3, 42.1, 38.4, 32.6, 31.2, 29.6, 21.2, 14.3; LC-MS (ESI+): t_R = 4.928 min, *m/z* 397.3 $[M+1]^+$; HRMS (TOF, ESI) *m/z*: $[M+H]^+$ calculated for $C_{22}H_{30}FN_6$:397.2516, found: 397.2529; HPLC: t_R = 8.860 min; purity: 97%.



1-((*trans*-4-Aminocyclohexyl)methyl)-3-(4-fluorophenyl)-*N*-(3-phenylpropyl)-1*H*-pyrazolo[3,4-d]pyrimidin-6-amine (44)

The title compound **44** (0.076 g, 68%) was prepared as a yellow solid according to general procedure F from **8c-3** (0.10 g, 0.19 mmol) and 4-fluorophenylboronic acid (0.050 g, 0.36 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 9.06 (s, 1H), 8.01–7.93 (m, 2H), 7.32–7.20 (m, 6H), 7.17 (t, *J* = 7.0 Hz, 1H), 4.17 (d, *J* = 6.8 Hz, 2H), 3.51 (t, *J* = 7.1 Hz, 2H), 3.08–2.96 (m, 1H), 2.75 (t, *J* = 7.5 Hz, 2H), 2.02 (dt, *J* = 14.5, 7.2 Hz, 5H), 1.81 (d, *J* = 12.4 Hz, 2H), 1.37 (qd, *J* = 12.6, 2.9 Hz, 2H), 1.22 (ddd, *J* = 15.4, 13.2, 2.6 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 165.9, 163.5, 157.6, 153.7, 145.3, 143.2, 130.0, 129.9, 129.5, 129.4, 126. 9, 117.0, 116.8, 52.2, 51.3, 42.0, 38.4, 34.3, 32.0, 31.2, 29.6; LC-MS (ESI): t_R = 5.046 min, *m/z* 459.3 $[M + 1]^+$; HRMS (TOF, ESI) *m/z*: $[M + H]^+$ calculated for $C_{27}H_{31}FN_6$: 458.2594, found:; HPLC: t_R = 9.225 min; purity: 99%.

Biological Methods

Microfluidic Capillary Electrophoresis (MCE) Assay

The activity assays were performed in a 384 well, polypropylene microplate in a final volume of 50 uL in 50 mM Hepes, Ph 7.4 containing 0.1% Bovine Serum Albumin (BSA), 0.1% Triton X-100, 10 mM $MgCl_2$ and ATP at the Km value for that enzyme (Table 3). All reactions were terminated by addition of 50 uL of 70 mM EDTA. Phosphorylated and unphosphorylated substrate peptides (Table 3) were separated following a 180 minute incubation on a LabChip EZ Reader equipped with a 12-sipper chip in separation buffer supplemented with CR-8 and analyzed using EZ Reader software.

Table 3. Assay conditions for MCE assays

Kinase	Peptide Substrate	ATP Concentration (uM)
Mer	EFPIYDFLPAKKK-$CONH_2$	27
Axl	KKKKEEIYFFF-$CONH_2$	97
Tyro	EFPIYDFLPAKKK-$CONH_2$	41

Ki Measurement

Inhibition of Mer kinase by **43** was measured in the MCE assay. Reactions (70 µL) were performed in V bottom polypropylene 384-well microplates containing 5 µL of compound in 10% DMSO and 1X Assay Buffer (50 mM Hepes pH 7.4, 10 mM $MgCl_2$, 0.01% Triton X-100, 0.1% BSA, and 1 mM DTT). An array in a 7 x 8 grid pattern was set up of **43** versus ATP. **43** was titrated starting at 1.5 times the IC_{50} (2.9 nM) and titrated downward 1.5x for 8 points. ATP was titrated starting at 4x the $ATPk_{m\ Apparent}$ (27µM) for a 3 fold - 7 point serial dilution. 22.5µL

of the ATP titration was transferred to the compound plate. A cocktail of Mer Kinase (2 nM) and Mer Peptide Substrate (Table 3)(1 μM) in 22.5μL was added to initiate the reaction. The reaction was allowed to proceed for 3.0 hr at room temperature and then terminated with EDTA (35 mM). The plate was read on a EZReader, using upstream voltage = -2250 V, downstream voltage = -500 V and pressure = -1.0 psi. The steady-state velocity was analyzed by linear regression of the Peptide in μM/min vs. ATP μM and plotted to determine Michaelis-Menten kinetics (GraphPad Prism 5.0). The concentration of **43** versus K_m values were then plotted to determine the relationship of the ATP and the inhibitor on enzyme kinetics (Figure 3).

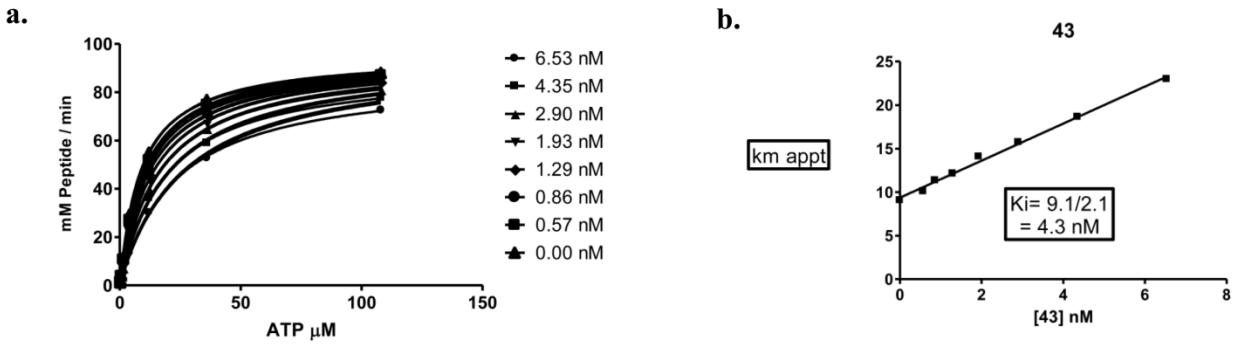


Figure 3. a. Michaelis-Menten kinetics of **43**; **b**. Ki determination plot of **43**.

Selectivity Profiling

The selected compounds were screened against 72 kinases using the Profiler Pro Kit (Caliper Life Sciences). Pre-plated enzyme stocks were reconstituted with 15 μL of supplied Reconstitution Buffer containing DTT and Protease Inhibitor Cocktail. 1 μL of compound at 26x of final concentration of compound in the reaction in 100% DMSO was transferred to the plate and the reaction was inhibited by the addition of substrate solution containing ATP and cofactors specific for each enzyme. The reaction was incubated at room temperature for 90 min. After

incubation 45 µL of termination buffer was added to stop the reaction and the plates were read in the Caliper EZ Reader.

Table 4.The selectivity profiling results of **42** and **43**[a].

Row Labels	Compound 42		Compound 43	
	10xIC50	100xIC50	10xIC50	100xIC50
ABL	6	32	5.5	49
AKT1	-0.5	1.5	-3	-4
AKT2	2	0.5	-4.5	-4
AMPKa1	6.5	38	9	62.5
AMPKAlpha2_Beta1_Gamma1	4.5	33.5	5.5	48.5
AurA	31	62	33.5	80.5
AXL_Caliper	28.5	80	13.5	67
BTK	-8	-4.5	-15.5	-8.5
CaMK2 Alpha	-4.5	-2.5	-4	-2.5
CAMK2gamma	-18	-13.5	-13	15
CaMK4	0.5	-0.5	-8	-4
CDK1/CycB1	0.5	0	-1.5	-3
CDK2	3	-1.5	-5.5	-4
CHK1	8	32.5	15	57.5
CHK2	0	1	4.5	24
CK1 Delta	2	-2	-4.5	1.5
CK1y3	-2	-2	-6.5	-7
c-TAK1	-1	10	-2	22
DYRK1a	4	1	-5	0.5
EGRF T790M	2.5	3.5	-5	-3.5
EPHB1	-0.5	4.5	-5	-4
Erk1	4.5	-2	-4	-5
Erk2	3	-3	-5	-5.5
FGFR1	5	30.5	3.5	37.5
FGFR3 K650E	5.5	21.5	-6.5	37.5
FLT3	82	95.5	81.5	98.5
FYN	3	6	0.5	9.5
GSK3Beta	0	-4	-3	4
HGK	-2	2.5	-2	4
IGFR1	0	-3	-9.5	-8
INSR	1.5	0.5	-4	-4.5
IRAK4	5	7.5	-1.5	9
ITK	5	25.5	-0.5	30.5
JAK2	-5.5	-3	-7.5	1
KDR	7	43.5	2	55.5
KIT	-13	-10	-8	-2
KIT T670I	-23.5	-21.5	-28.5	-19

LCK	1.5	18	-1.5	2.5
LYNa	1.5	14.5	-3	23
MAPKAPK2	94.5	86	92	87.5
MAPKAPK5	0.5	-6.5	-2.5	3
MARK1	8	37.5	10.5	53
MARK4	14	60	17.5	70
MELK	36	89.5	30.5	83.5
Mer_Caliper	74.5	97	74.5	97.5
MET	4	4.5	-2	10
MET M1250T	-1.5	-1	-1	-1
MSK1	2	1	-4	2
MST2	2.5	3.5	2.5	30
P38 Alpha	3	0.5	1.5	21
P70S6K	2.5	5.5	3.5	9.5
PAK2	-3.5	-2	0.5	3
PDK2	2.5	-1	-5	-8
PIM2	-5.5	-5.5	-7.5	-5.5
PKAC Alpha	-2	1	0	14
PKC Zeta	-13.5	17	-10.5	22.5
PKCB2	-7	-28.5	2.5	2
PKCG1alpha	3	4	-1.5	-1.5
PKCi	-3.5	2.5	-0.5	-4
Raf1	5	2.5	-3	-6
Ret	79	99.5	59	96
RET Y791F	67	95	56	93
Rock2	-5	-4.5	0	3.5
ROS	1	6	2	2
RSK1	10	34	18.5	69.5
SGK	1	1.5	-4.5	-1
SRC	3.5	13	6	11
SYK	-0.5	-1.5	-5.5	-5
TRKC	31	76.5	31	80.5
TXK	-4	18	-10	8
Tyro3_Caliper	9	46	30.5	81.5
ZIPK	1.5	4	-1.5	8

[a] An average result from duplicate runs.

697-Cell Based Assay for Mer Kinase Inhibition:

$5x10^6$ 697 cells were cultured in 1 ml RPMI containing 10% fetal bovine serum (FBS). Pervanadate solution was prepared by combining 20 mM sodium orthovanadate in 0.9x PBS in a 1:1 ratio with 0.3% (w/w) hydrogen peroxide in PBS for 15 minutes at room temperature.

Freshly prepared pervanadate (12 µL per well) was added to cultures for 1 minute at room temperature. Cells were collected and lysates were prepared in 120 µL lysis buffer (50 mM HEPES pH 7.5, 150 mM NaCl, 10 mM EDTA, 10% glycerol, and 1% Triton X-100) supplemented with protease inhibitors (Complete mini, Roche Molecular Biochemicals). Mer protein was immunoprecipitated with 0.9 µg α-Mer monoclonal antibody (R&D Systems, #MAB8912) and 15 µL 50% Protein G beads in PBS (InVitrogen) at 4˚C overnight. Samples were analyzed by SDS-PAGE and immunoblotting with a custom polyclonal antibody that recognizes the phosphorylated form of Mer. Antibody was stripped from the membrane by incubating in (STRIPPING SOLN) at 60 ºC for 40 minutes with agitation. Total Mer protein was then detected with an α-Mer polyclonal antibody (Epitomics Inc., #1633-1). Proteins were visualized by enhanced chemoluminescence and relative levels were quantitated using Quantity One software (Bio-Rad). IC_{50} values were determined by non-linear regression using Graphpad Prism v4 software (Graphpad Software, Inc). In this cell-based assay, the IC_{50} for inhibition of phospho-Mer by **43** was 141 nM with a 95% confidence interval of 110.5 to 178.9 nM (Figure 4).

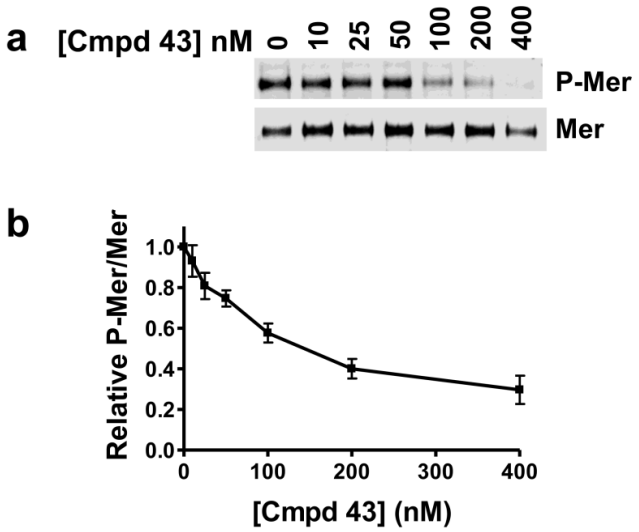


Figure 4. Compound **43** inhibits accumulation of activated Mer protein in acute leukemia cells. 697 cell cultures were treated with the indicated concentrations of **43** for 1 hour. Pervanadate was added to cultures for 1 minute to stabilize the phosphorylated form of Mer. Mer was immunoprecipitated from cell lysates and total Mer protein and Mer phosphoprotein were detected by immunoblot. **a.** Representative western blots. **b**. Relative levels of phospho-Mer and Mer proteins were determined. Mean values +/- standard error derived from 4 independent experiments are shown.

Protein Expression and Purification

A pET28 expression vector containing the coding sequence for the Mer catalytic domain[1] was transformed into Rosetta BL21(DE3)pLysS competent cells (Novagen). Cells were grow at 37°C with shaking until the OD_{600} reached ~0.8 at which time the temperature was lowered to 15°C and expression was induced by adding 0.2 mM IPTG and continuing shaking overnight. Cells were harvested by centrifugation and pellets were stored at -80 °C. Cell pellets were thawed and resuspended in 30ml of lysis buffer (50 mM sodium phosphate pH 7.2, 50 mM NaCl, 30 mM imidazole, 1X protease inhibitor cocktail (Roche)) per liter of culture. Cells were lysed on ice by sonication at 40% amplitude for a total of 15 minutes consisting of 10 second pulses followed by 50 second rest. The cell lysate was clarified by centrifugation and loaded onto a HisTrap FF column (GE Healthcare) that had been preequilibrated with 10 column volumes of binding buffer (50 mM sodium phosphate pH 7.2, 500 mM NaCl, 30 mM imidazole) using an AKTA FPLC (GE Healthcare). The column was washed with 15 column volumes of binding buffer and protein was eluted in a linear gradient to 100% elution buffer (50 mM sodium phosphate pH 7.2, 500 mM NaCl, 500 mM imidazole) over 20 column volumes. Peak fractions

containing Mer were pooled and concentrated to 2.0 mL in Amicon Ultra-15 concentrators, 10,000 molecular weight cut-off (Millipore). Concentrated Mer was loaded onto a HiLoad 26/60 Superdex 200 prep grade column (GE Healthcare) that had been preequilibrated with 1.2 column volumes of sizing buffer (20 mM Tris pH 8.0, 500 mM NaCl, 2.0 mM β-mercaptoethanol) using an ATKA FPLC (GE Healthcare). Protein was eluted isocratically in sizing buffer over 1.3 column volumes at a flow rate of 2.0 mL/min collecting 3.0 mL fractions. Peak fractions were analyzed for purity by SDS-PAGE and those containing pure Mer were pooled and concentrated using Amicon Ultra-15 concentrators 10,000 molecular weight cut-off (Millipore) to a final concentration of 35-40 mg/mL.

Crystallization

Crystals of Mer in complex with **43** were obtained by vapor diffusion from sitting drops at 12 °C. Protein at 32.5 mg/mL in crystallization buffer (20 mM Tris pH 8.0, 500mM sodium chloride, 2mM β-mercaptoethanol) was incubated with **43** (dissolved in DMSO) to give a final concentration of 2.5 mM and slowly rocked over night. This solution was mixed 1:1 with and equilibrated against crystallization solution containing 27-33% (v/v) Peg 400, 200 mM magnesium chloride, 100mM Tris pH 8.5. Plate-like crystals grew to final dimensions of up to 1000 x 400 x 50 μm over 10 days. Prior to diffraction data collection, crystals were vitrified by plunging into liquid nitrogen. The Mer/**43** crystals displayed the symmetry of space group $P2_1$ with cell parameters a = 51 Å, b =91 Å, c = 68 Å, β=100.28°, contained two molecules in the asymmetric unit, and diffracted X-rays to a minimum Bragg spacing of about 2.69 Å.

Structure Determination

Data were collected at the Southeast Regional Collaborative Access Team (SER-CAT) 22-ID beamline at the Advanced Photon Source, Argonne National Laboratory at a wavelength of 1.0621Å and a temperature of 100K. Data were processed using the program HKL2000. The diffraction quality of our crystals was non-uniform: when the thin edge was exposed to X-rays, the resulting diffraction spots were radially smeared, which was accompanied by a loss of resolution and a somewhat reduced completeness in the high-resolution shells.

The structure of the Mer/**43** complex was determined by molecular replacement with the program Phaser[2]. The search model was generated from the coordinates of Mer in complex with ADP (PDB entry 3BRB) with all non-protein atoms removed. Refinement was carried out using the program Refmac[3] from the CCP4 suite[4], interspersed with manual revisions of the model using the program Coot[5]. Refinement consisted of conjugate-gradient minimization and calculation of individual atomic displacement and translation/libration/screw (TLS) parameters. To avoid any model bias, coordinates for **43** were not included until the remainder of the model (including water molecules and ions) was completed. Inclusion of high-resolution, albeit weaker, data increased the stability and convergence of the refinement process. For data collection and refinement statistics see Table 5.

The current model contains two molecules of the Mer kinase domain bound to compound **43**, 3 chloride ions, 3 calcium ions, and 36 water molecules. Residues that could not be identified in the electron density were: chain A, 552-576, 595-599, 659-664, 743-762, 864; chain B, 552-575, 596-598, 623-636, 743-761, 862-864. The model exhibits excellent geometry as determined by MolProbity.[6] A Ramachandran analysis identified 95.7% favored, 4.1% allowed, and 0.2% disallowed residues. The coordinates and structure factors have been deposited in the RCSB Protein Data Bank under accession number 3TCP.

Table 5. Data collection and refinement statistics

Data collection	
Space group	$P2_1$
Cell dimensions *a*, *b*, *c* (Å), β(°)	51.2, 91.1, 68.4, 100.35
Resolution (Å)	29.2 – 2.69 (2.72 – 2.69)
R_{merge} (%)[a]	9.8 (52.2)
$I/\sigma I$	14.7 (1.5)
Unique reflections	16,603 (252)
Completeness (%)	96.3 (59.3)
Redundancy	3.6 (2.0)
Wilson B-factor (Å^2)	63.2
Refinement	
Resolution (Å)	28.88 – 2.69 (2.76 – 2.69)
No. of reflections (work/free)	15,728/840 (913/50)
Cut-off (σ)	0
R_{work} / R_{free}	22.5/29.8 (25.4/43.3)
No. of atoms	
Protein	4,059
Compound 43	58
Ions	6
Water	36
B-factors (Å^2)	
Protein	57.8
Compound 43	48.4
Ions	63.4
Water	53.3
R.m.s. deviations	
Bond lengths (Å)	0.008
Bond angles (°)	1.147
Ramachandran[b]	
Favored (%)	95.7
Generally Allowed (%)	4.1
Disallowed (%)	0.2
Missing residues	A: 552-576, 595-599, 659-664, 743-762, 864 B: 552-575, 596-598, 623-636, 743-761, 862-864

Values in parentheses denote highest resolution shell

[a] $R_{merge} = 100\ \Sigma_h\Sigma_i|I_{h,i} - \langle I_h\rangle|/\Sigma_h\Sigma_i I_{h,i}$, where the outer sum (h) is over the unique reflections and the inner sum (i) is over the set of independent observations of each unique reflection.
[b] As defined by the validation suite MolProbity[6].

DMPK Study

A group of 9 male Swiss albino mice (group I) were dosed intravenously (IV) with solution formulation of **43** in 7.5 % (v/v) *N*-methyl pyrrolidone and 40% (v/v) PEG in water. Another group of 9 male Swiss albino mice (group II) were dosed orally (PO) with suspension formulation of **43** in 0.5% (w/v) NaCMC with 0.1% (v/v) Tween-80 in water. From each mouse, three blood samples (60 μL) were collected from retro orbital plexus such that samples were obtained at 0, 0.08, 0.25, 0.5, 1, 2, 4, 8 & 24 h (iv) & 0, 0.25, 0.5, 1, 2, 4, 6, 8 & 24 h (po) post dose. At each time point blood samples were collected from three mice. Immediately after collection, plasma was harvested by centrifugation of blood and was stored below -70ºC until analysis. All samples were processed for analysis by precipitation using Albendazole as internal standard and analyzed with partially validated LC/MS-MS method (LLOQ was 1.038 ng/mL). Pharmacokinetic parameters were calculated using the Non-compartmental analysis tool of WinNonlin® Enterprise software (version 5.2) (Table 6).

Table 6. PK profile of **43**

Route[a]	T1/2 (h)	Tmax (h)	Cmax (ng/mL)	AUClast (h*ng/mL)	Cl_obs (mL/min/Kg)	Vss (L/Kg)	%F
IV	4.41	0.083	557	2522	19.5	5.83	-
PO	-	4.0	122	1427	-	-	57

[a] a dose of 3mg/Kg for both routes; n = 9 mice per group.

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